Pyrazolopyrimidines Establish MurC as a Vulnerable Target in *Pseudomonas aeruginosa* and *Escherichia coli*

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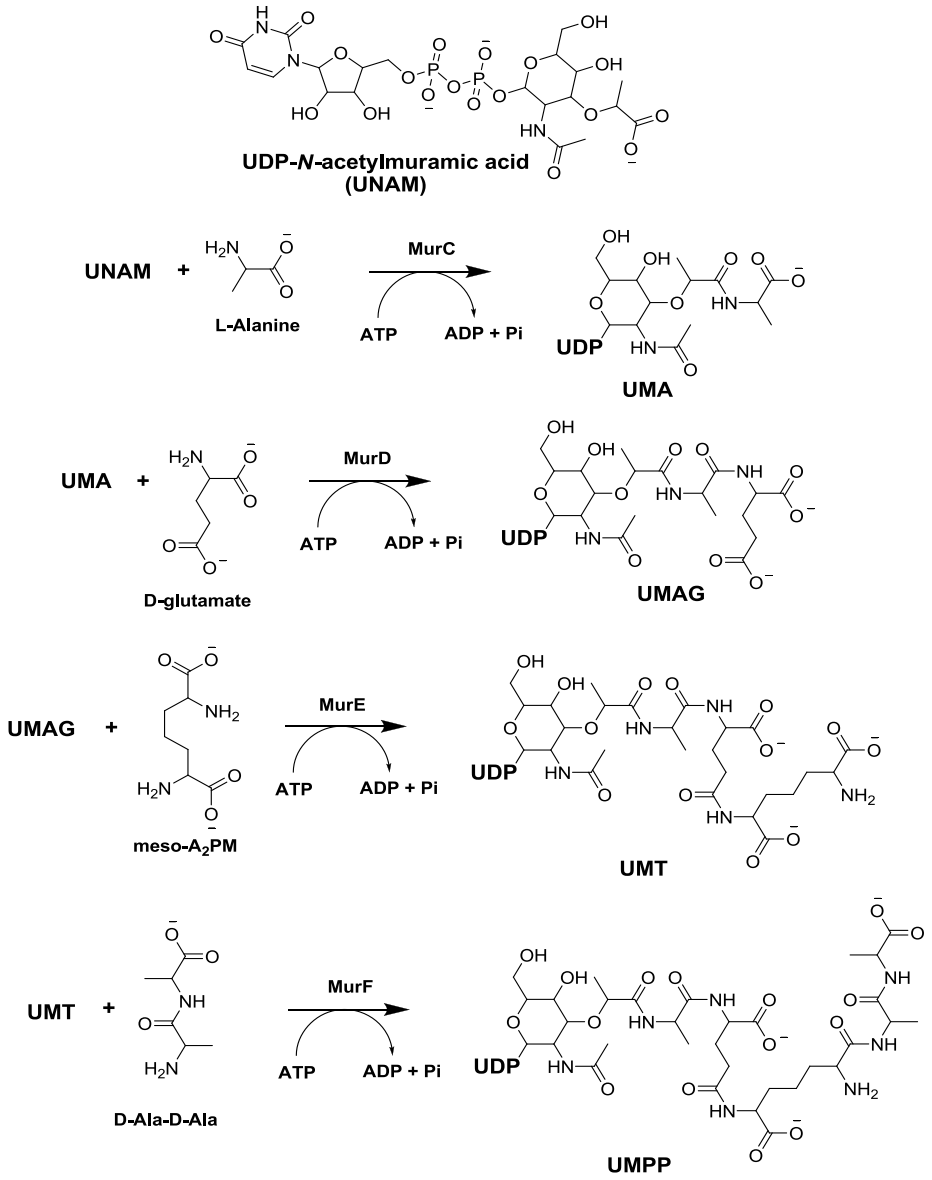
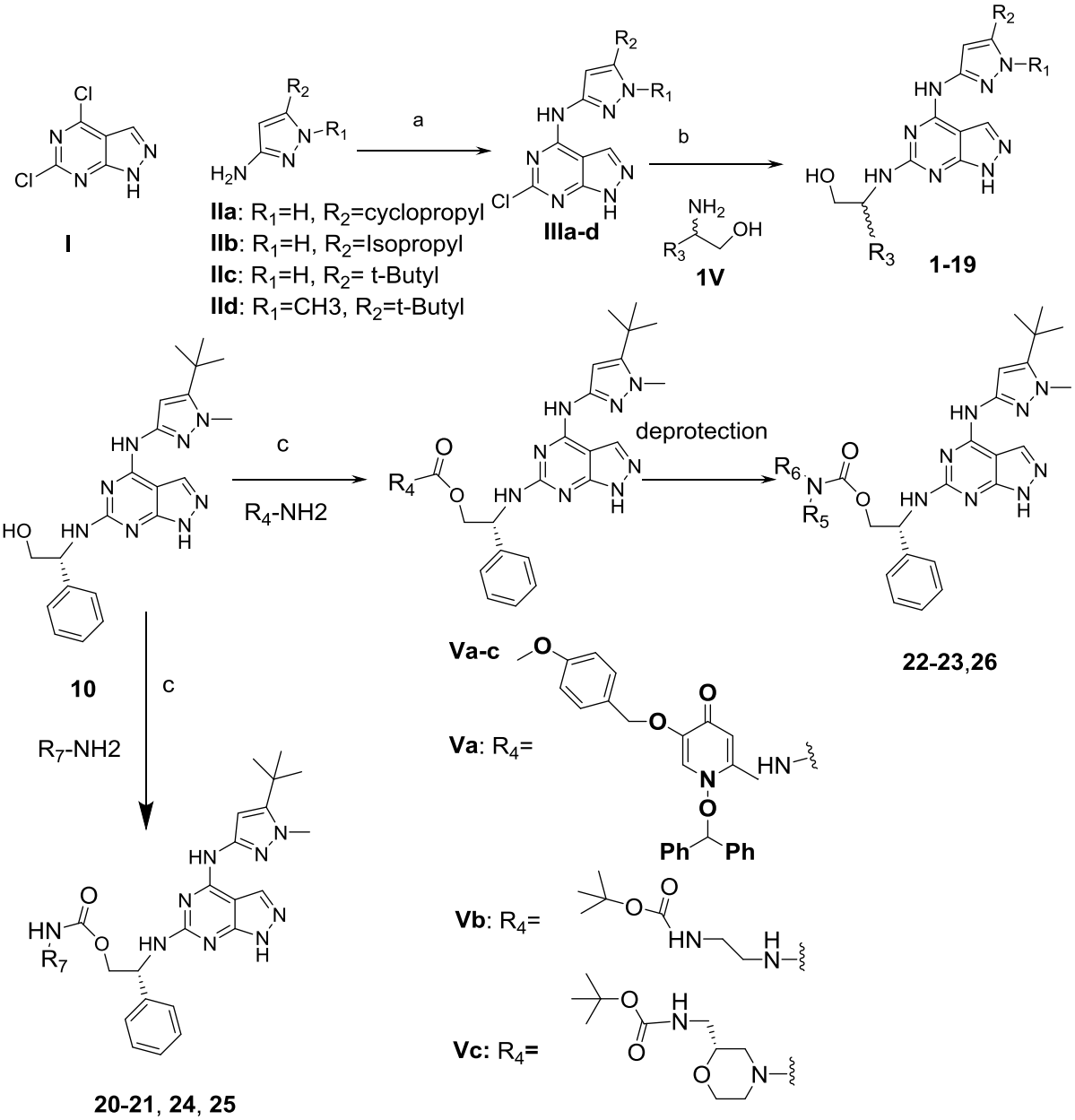


Figure S1: Reactions catalysed by Mur ligases. UNAM: UDP-N-acetylmuramic acid; UMA: UDP-*N*-acetylmuramic acid; UMAG: UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate; UMT: UDP-*N*-acetylmuramoyl tripeptide; UMPP: UDP-*N*-acetylmuramoyl:pentapeptide [Hilgers, L, W., Tari, R. A., Wijnands, M, W., Knuth, C. D., Mol, A., Brooun, D. R., Dougan, M. T. (2003) Crystal Structures of Active Fully Assembled Substrate- and Product-Bound Complexes of UDP-*N*-Acetylmuramic Acid:L-Alanine Ligase (MurC) from *Haemophilus influenzae J. Bact. 185*, 4152–4162]

S2 – Chemistry

Scheme 1



Reagents : (a) Na_2CO_3, Ethanol, 80 ^oC(b) Na_2CO_3/DIPEA, n-Butanol, 160-170 ^oC, MW (c) CDI, DIPEA/DCM, RT to 50 ^oC

Intermediate IIIa

6-chloro-N-(3-cyclopropyl-1H-pyrazol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

In a 250 ml round bottom flask, 3-cyclopropyl-1H-pyrazol-5-amine (3.3 g, 26.4 mmol) and sodium carbonate (5.6 g, 52.9 mmol) were taken in ethanol (100 ml). Then, 4,6-dichloro-1H-pyrazolo[3,4-d]pyrimidine (5.00 g, 26.4 mmol, **Activate Scientific GmbH Chemistry**) was added and heated at 80 °C for 12 h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. Reaction mixture was filtered and washed with methanol and organic layer was concentrated under vacuum. The crude solid obtained was triturated with water, filtered, washed with water and dried to afford title compound (7.0 g, 96 %). MS (ES+), (M+H)+ = 276.2 for $C_{11}H_{10}ClN_7$

Intermediate IIIb

6-chloro-N-(3-isopropyl-1H-pyrazol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine

In a 250 ml RB, 3-isopropyl-1H-pyrazol-5-amine (0.695 g, 5.56 mmol) and sodium carbonate (1.122 g, 10.58 mmol) were taken in ethanol (40 ml). Then, 4,6-dichloro-1H-pyrazolo[3,4-d]pyrimidine (1.0 g, 5.29 mmol) was added and heated at 80 °C for 2 h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. Reaction mixture was filtered washed with methanol and organic layer was concentrated under vacuum. The crude solid obtained was triturated with water, filtered, washed with water and dried to afford title compound (1.80 g, quantitative).
MS (ES+), (M+H)+ = 278.21 for $C_{11}H_{12}ClN_7$

Intermediate IIIc

N-(3-tert-butyl-1H-pyrazol-5-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine

In a 250 ml RB, 3-tert-butyl-1H-pyrazol-5-amine (0.773 g, 5.56 mmol) and sodium carbonate (1.122 g, 10.58 mmol) were taken in ethanol (40 ml). Then, 4,6-dichloro-1H-pyrazolo[3,4-d]pyrimidine (1.0 g, 5.29 mmol) was added and heated at 80 °C for 2h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. Reaction mixture was filtered, washed with methanol and organic layer was concentrated under vacuum. The crude solid obtained was triturated with water, filtered, washed with water and dried to afford title compound (1.50 g, 97%).
MS (ES+), (M+H)+ = 292.27 for $C_{12}H_{14}ClN_7$

Intermediate IIId

N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine

In a 100 ml round bottom flask, 5-tert-butyl-1-methyl-1H-pyrazol-3-amine (0.446 g, 2.91 mmol, Aurora Fine Chemicals LLC) and sodium carbonate (0.617 g, 5.82 mmol) were taken in ethanol (40 ml). Then, 4,6-dichloro-1H-pyrazolo[3,4-d]pyrimidine (0.550 g, 2.91 mmol) was added and heated at 80 °C for 2 h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. Reaction mixture was filtered,

washed with methanol and organic layer was concentrated under vacuum. The crude solid obtained was triturated with water, filtered, washed with water and dried to afford title compound (0.460 g, 51.7%).
MS (ES+), (M+H)+ = 306.33 for $C_{13}H_{16}ClN_7$

(1-(4-(3-cyclopropyl-1H-pyrazol-5-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)piperidin-2-yl)methanol (1)

In a 10 ml CEM microwave vial, 2-piperidinemethanol (0.074 g, 0.64 mmol, Aldrich) ,6-chloro-N-(3-cyclopropyl-1H-pyrazol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (0.160 g, 0.58 mmol, **IIIa**) and sodium carbonate (0.123 g, 1.16 mmol) were taken in butan-1-ol (5 ml).The reaction mixture was subjected to microwave irradiation at 160 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. The reaction mixture was filtered, washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.037 g, 18 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.56 - 0.77 (m, 2 H), 0.78 - 1.05 (m, 2 H), 1.31 - 1.54 (m, 2 H), 1.54 - 1.76 (m, 3 H), 1.78 - 2.00 (m, 2H), 2.87 (t, *J*=12.0 Hz, 1 H), 3.39 - 3.54 (m, 1 H), 3.68 (td, *J*=9.5, 5.18 Hz, 1 H), 4.65 (br. s., 1 H), 4.60 (br. s., 1 H), 4.83(br. s., 1 H), 6.12 - 6.53 (m, 1 H), 7.68 - 8.27 (m, 1 H), 10.03 (br. s., 1 H), 12.08 (br. s., 1 H), 12.54 (br. s., 1 H); HRMS: *m/z* (ES+); calculated, 355.19166; found, 355.19923 (MH^+) for $C_{17}H_{22}N_8O$.

(S)-2-cyclohexyl-2-(4-(3-cyclopropyl-1H-pyrazol-5-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)ethanol (2)

In a 10 ml CEM microwave vial, (S)-2-amino-2-cyclohexylethanol (0.069 g, 0.48 mmol, S.D Fine), 6-chloro-N-(3-cyclopropyl-1H-pyrazol-5-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (0.120 g, 0.44 mmol, **IIIa**), sodium carbonate (0.092 g, 0.87 mmol) and DIPEA were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 160 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. The reaction mixture was filtered, washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.032 g, 19.22 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.61 - 0.77 (m, 2 H) 0.81 - 0.98 (m, 2 H) 0.96 - 1.38 (m, 5 H) 1.52 - 1.86 (m, 6 H) 3.48 - 3.62 (m, 2 H) 3.71 - 3.97 (m, 3 H) 4.37 - 4.83 (m, 1 H) 5.87 - 6.64 (m, 1 H) 7.84 - 8.14 (m, 1 H) 8.16 - 8.26 (m, 1 H) 9.91 - 10.49 (m, 1 H) 12.28 - 12.90 (m, 1 H); HRMS: *m/z* (ES+); calculated, 383.22296; found, 383.22965 (MH^+) for $C_{19}H_{26}N_8O$.

(S)-2-cyclohexyl-2-(4-(5-isopropyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)ethanol (3)

In a 10 ml CEM microwave vial, (S)-2-amino-2-cyclohexylethanol (85 mg, 0.59 mmol, Chemlabs), 6-chloro-N-(5-isopropyl-1H-pyrazol-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (150 mg, 0.54 mmol, **IIIb**), sodium carbonate (0.114 g, 1.08 mmol) and DIPEA (0.094 ml, 0.54 mmol) were taken in butan-1-ol (5 ml).The RM was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. The reaction mixture was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.060 g, 24.99 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.81 - 1.16 (m, 4 H) 1.34 (d, J=6.8 Hz, 6 H)1.47 - 1.86 (m, 5 H) 1.89 (s, 3H) 2.80 - 3.04 (m, 1 H) 3.44 - 3.68 (m, 2 H) 3.70 - 4.07 (m, 1 H) 4.42 - 4.73 (m, 1 H) 5.91 - 6.99 (m, 1 H) 5.94 - 6.83 (m, 1 H) 7.62 - 8.23 (m, 1 H) 7.77 - 8.21 (m, 1 H) 9.68 - 10.40 (br. s., 1 H) 9.99 - 10.47 (br. s., 1 H) 11.67 - 12.01 (br. s., 1 H) 12.15 - 12.95 (br. s., 1 H); HRMS: *m/z* (ES+); calculated, 385.23861, found 385.24556 (MH^+) for $C_{19}H_{28}N_8O$·[Acetic acid].

(S)-2-(4-(5-tert-butyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-cyclohexylethanol (4)

In a 10 ml CEM microwave vial, (S)-2-amino-2-cyclohexylethanol (81 mg, 0.57 mmol, Chemlabs), N-(5-tert-butyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (150 mg, 0.51 mmol, **IIIc**), sodium carbonate (0.109 g, 1.03 mmol) and DIPEA (0.090 ml, 0.51 mmol) were taken in butan-1-ol (5 ml). The reaction mixture was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.060 g, 25.4 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.81 - 1.19 (m, 5 H) 1.29 (s, 9 H) 1.42 - 1.85 (m, 6 H) 1.89 (s, 3 H) 3.52 (d, *J*=3.4 Hz, 2 H) 3.68 - 4.06 (m, 1 H) 4.27 - 4.80 (br. s, 1 H) 5.86 - 6.37 (br. s, 1 H) 6.45 - 7.02 (br. s, 1 H) 7.71 - 8.21 (br. s, 1 H) 9.86 - 10.52 (br. s, 1 H) 11.67 - 12.26 (br. s, 1 H) 12.32 - 12.85 (br. s, 1 H); HRMS: *m/z* (ES+); calculated, 399.25426, found, 399.26089 (MH^+) for $C_{20}H_{30}N_8O$·[Acetic acid].

(R)-2-cyclohexyl-2-(4-(3-isopropyl-1H-pyrazol-5-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)ethanol (5)

In a 10 ml CEM microwave vial, (R)-2-amino-2-cyclohexylethanol (85 mg, 0.59 mmol, Chemlabs), 6-chloro-N-(5-isopropyl-1H-pyrazol-3-yl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine (150 mg, 0.54 mmol, **IIIb**), sodium carbonate (114 mg, 1.08 mmol), and DIPEA (0.094 ml, 0.54 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was

monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.025 g, 12.04 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.90 - 1.20 (m, 5 H) 1.37 (d, J=6.8 Hz, 6 H) 1.51 - 1.72 (m, 4 H) 1.73 - 1.87 (m, 2 H) 2.90 - 3.01 (m, 1H) 3.46 - 3.57 (m, 2 H) 3.91 - 4.01 (m, 1H) 4.52 - 4.66 (m, 1 H) 6.35 - 6.55 (m, 1 H) 7.96 - 8.13 (br. s, 1 H) 10.02 - 10.43 (br. s, 1 H) 11.95 – 12.15 (br. s, 1 H) 12.33 - 12.63 (br. s, 1 H); HRMS: *m/z* (ES+); calculated, 385.23861, found, 385.24538 (MH^+) for $C_{19}H_{28}N_8O$.

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-cyclohexylethanol (6)

In a 10 ml CEM microwave vial, (R)-2-amino-2-cyclohexylethanol (73.8 mg, 0.52 mmol, Chemlabs), N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (150 mg, 0.49 mmol, **IIId**), sodium carbonate (104 mg, 0.98 mmol), and DIPEA (0.086 ml, 0.49 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.034 g, 16.80 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.90 - 1.27 (m, 5 H) 1.37 (s, 9 H) 1.51 - 1.73 (m, 4 H) 1.73 - 1.87 (m, 2 H) 3.46 - 3.57 (m, 2 H) 3.84 (s, 3 H) 3.87 - 3.98 (m, 1 H) 4.42 - 4.66 (m, 1 H) 6.03 - 6.27 (m, 1 H) 6.73 (s, 1 H) 7.86 - 8.13 (br. s, 1 H) 10.02 - 10.23 (br. s, 1 H) 12.33 - 12.63 (br. s, 1 H); HRMS: *m/z* (ES+); calculated, 413.26991, found, 413.27721 (MH^+) for $C_{21}H_{32}N_8O$.

((S)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-cyclohexylethanol (7)

In a 10 ml CEM microwave vial, (S)-2-amino-2-cyclohexylethanol (73.8 mg, 0.52 mmol, Chemlabs), N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (150 mg, 0.49 mmol**,** **IIId**), sodium carbonate (104 mg, 0.98 mmol), and DIPEA (0.086 ml, 0.49 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.030 g, 14.82 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.88 - 1.26 (m, 5 H) 1.37 (s, 9 H) 1.52 - 1.72 (m, 4 H) 1.73 - 1.85 (m, 2 H) 3.53 (d, *J*=3.8 Hz, 2 H) 3.84 (s, 3 H) 3.87 - 4.00 (m, 1 H) 4.41 - 4.70 (m, 1 H) 6.09 - 6.29 (br. s, 1 H)

6.75 (s, 1 H) 7.89 - 8.17 (br. s, 1 H) 9.84 - 10.26 (br. s, 1 H) 12.24 - 12.73 (br. s, 1 H); HRMS: *m/z* (ES+); calculated, 413.26991, found 413.27734 (MH^+) for $C_{21}H_{32}N_8O$.

2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-(tetrahydro-2H-pyran-4-yl)ethanol (8)

In a 10 ml CEM microwave vial, 2-amino-2-(tetrahydro-2H-pyran-4-yl)ethanol (78 mg, 0.54 mmol, PharmaCore), N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (150 mg, 0.49 mmol, **IIId**), sodium carbonate (104 mg, 0.98 mmol), and DIPEA (0.086 ml, 0.49 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.048 g, 20.62 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.21 - 1.32 (m, 2 H) 1.37 (s, 9 H) 1.58 - 1.72 (m, 2 H) 1.89 (s, 3H) 3.13 - 3.30 (m, 4 H) 3.50 - 3.59 (m, 3 H) 3.84 (s, 3 H) 3.85 - 3.97 (m, 2 H) 6.27 (d, *J*=9.2 Hz, 1 H) 6.66 (s, 1 H) 7.83 - 8.11 (br. s, 1 H) 9.88 - 10.15 (br. s, 1 H) 12.26 - 12.70 (br.s, 1 H); HRMS: m/z (ES+); calculated 415.25616, found 415.25605 (MH+) for $C_{20}H_{30}N_8O_2$•[Acetic acid].

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-3-methylbutan-1-ol (9)

In a 10 ml CEM microwave vial, (R)-2-amino-3-methylbutan-1-ol (74.2 mg, 0.72 mmol, Alfa Aesar) N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (200 mg, 0.65 mmol, **IIId**), sodium carbonate (139 mg, 1.31 mmol), and DIPEA (0.114 ml, 0.65 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.022 g, 9.03 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.91 (t, J=5.9 Hz, 7 H) 1.37 (s, 9 H) 1.92 - 2.02 (m, 1 H) 3.47 - 3.58 (m, 2 H) 3.84 (s, 3 H) 3.87 - 3.96 (m, 1 H) 4.44 - 4.67 (m, 1 H) 6.14 - 6.29 (m, 1 H) 6.74 (s, 1 H) 7.92 - 8.12 (m, 1 H) 9.90 - 10.25 (m, 1 H) 12.36 - 12.71 (m, 1 H); HRMS: *m/z* (ES+); calculated, 373.23861, found 373.24599 (MH^+) for $C_{18}H_{28}N_8O$.

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (10)

In a 10 ml CEM MW vial, (R)-2-amino-2-phenylethanol (74.0 mg, 0.54 mmol, Alfa Aesar), N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (150 mg, 0.49 mmol, **IIId**), sodium carbonate (52 mg, 0.49 mmol), and DIPEA (0.086 ml, 0.25 mmol) were taken in butan-1-ol (5 ml). The RM was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.028 g, 14.04 %); ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.36 - 1.43 (s, 9 H) 3.60 - 3.74 (m, 2 H) 3.87 (s, 3 H) 4.92 (br. s., 1 H) 5.14 (br. s., 1 H) 6.67 (br. s.,1 H) 6.95 (d, *J*=8.7 Hz, 1 H) 7.13 - 7.22 (m, 1 H) 7.28 (t, *J*=7.4 Hz, 2 H) 7.34 - 7.43 (m, 2 H) 8.02 (br. s., 1 H) 10.05 (br. s., 1 H) 12.57 (br. s., 1H); HRMS: *m/z* (ES+); calculated, 407.23311, found 407.2302 (MH^+) for $C_{21}H_{26}N_8O$.

(S)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (11)

In a 10 ml CEM microwave vial, (R)-2-amino-2-phenylethanol (74.0 mg, 0.54 mmol, Alfa Aesar), N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (164mg, 0.54 mmol, **IIId**), sodium carbonate (114 mg, 1.07 mmol), and DIPEA (0.047 ml, 0.27 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.038 g, 17.43 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.40 (s, 9 H) 3.69 (t, *J*=5.6 Hz, 2 H) 3.79 - 3.88 (m, 3 H) 4.91 (br. s., 1 H) 5.14 (br. s., 1 H) 6.65 (br. s., 1 H) 6.94 (d, *J*=8.7 Hz, 1 H) 7.12 - 7.23 (m, 1 H) 7.28 (t, *J*=7.3 Hz, 2 H) 7.34 - 7.44 (m, 2 H) 8.01 (br. s., 1 H) 10.05 (br. s., 1 H) 12.57 (br. s., 1 H); HRMS: *m/z* (ES+); calculated, 407.22296, found, 407.2305 (MH^+) for $C_{21}H_{26}N_8O$.

(S)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-(3-methylisoxazol-5-yl)ethanol (12)

In a 10 ml CEM microwave vial, (S)-2-amino-2-(3-methylisoxazol-5-yl)ethanol (0.070 g, 0.49 mmol, AZ Strategic Reagent Collection), (5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (0.15 g, 0.49 mmol, **IIId**), sodium carbonate (104 mg, 0.98 mmol), and DIPEA (0.043 ml, 0.25 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO

and purified by reverse phase HPLC (Gilson) to afford title product (0.038 g, 18.83 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.37 (br. s., 9 H) 2.16 (s, 3 H) 3.71 - 3.81 (m, 2 H) 3.83 (s, 3 H) 5.12 (br. s., 1 H) 5.31 (br. s., 1 H) 6.17 (br.s., 1 H) 7.02 (br. s., 1 H) 8.07 (br. s., 1 H) 10.15 (br. s., 1 H) 12.68 (br. s., 1 H); HRMS: *m/z* (ES+); calculated, 412.21312, found, 412.22072 (MH^+) for $C_{19}H_{25}N_9O_2$.

(1R,2S)-1-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2,3-dihydro-1H-inden-2-ol (13)

In a 10 ml CEM microwave vial, (1R,2S)-1-amino-2,3-dihydro-1H-inden-2-ol (78 mg, 0.52 mmol, Aldrich), N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (160 mg, 0.52 mmol, **IIId**), sodium carbonate (111 mg, 1.05 mmol), and DIPEA (0.091 ml, 0.52 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.030 g, 13.7 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 0.80-1.52 (s, 9H), 2.67-2.93 (m, 1H), 2.92-3.12 (m, 1H), 3.80 (s, 3H), 4.56 (d, *J*=4.3 Hz, 1H), 5.18 (d, *J*=4.1 Hz,1H), 5.49 (br. s., 1H), 5.93-6.76 (m, 2H), 7.10-7.34 (m, 4H), 7.87-8.23 (m, 1H), 10.33 (br. s., 1H), 12.66 (br. s., 1H); HRMS: *m/z* (ES+); calculated, 419.22296, found,419.23006 (MH^+) for $C_{21}H_{27}N_9O_2$.

(R)-2-((4-((5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)amino)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)-2-(pyridin-2-yl)ethanol (14)

In a 10 ml CEM microwave vial, (R)-2-amino-2-(pyridin-2-yl)ethanol (54 mg, 0.39 mmol, Net Chem Inc.), N-(5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (120 mg, 0.39 mmol, **IIId**), sodium carbonate (83 mg, 0.78 mmol), and DIPEA (0.034 ml, 0.39 mmol) were taken in butan-1-ol (5 ml). The reaction mixture (RM) was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.015g, 9.38 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.38 (s, 9 H) 3.75 (br. s., 1 H) 3.79 - 3.94 (m, 5 H) 4.93 (br. s., 1 H) 5.17 (br. s., 1 H) 6.57 (s, 1 H) 6.85 (br. s., 1 H) 7.15 - 7.28 (m, 1 H) 7.37 (d, *J*=7.3 Hz, 1 H) 7.60 - 7.76 (m, 1 H) 8.04 (br. s., 1 H) 8.51 (br. s., 1 H) 10.12 (br. s., 1 H) 12.62 (br. s., 1 H); HRMS: *m/z* (ES+); calculated, 408.21821, found, 408.22594 (MH^+) for $C_{20}H_{25}N_9O$.

(R)-2-((4-((5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)amino)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)-2-(pyridin-3-yl)ethanol(15)

In a 10 ml CEM microwave vial, (R)-2-amino-2-(pyridin-3-yl)ethanol (87 mg, 0.63 mmol, NetChem Inc.), N-(5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (160 mg, 0.52 mmol, **IIId**), sodium carbonate (111 mg, 1.05 mmol), and DIPEA (0.091 ml, 0.52 mmol) were taken in butan-1-ol (5 ml). The RM was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.006g, 2.81 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.36 - 1.47 (s, 9H), 3.62 - 3.78 (m, 2H), 3.84 (s, 3H), 5.01 (br. s., 1H), 5.15 (br. s., 1H), 6.43 - 6.80 (m, 1H), 7.03 (d, *J*=9.0Hz, 1H), 7.32 (dd, *J*=7.8, 4.99 Hz, 1H), 7.78 (d, *J*=7.9 Hz, 1H), 8.02 (br. s., 1H), 8.40 (d, *J*=3.4 Hz, 1H), 8.59 (s, 1H), 10.07 (br. s., 1H), 12.38 - 12.77 (br. s,1H); HRMS: *m/z* (ES+); calculated, 408.22621, found, 408.22522 (MH^+) for $C_{20}H_{25}N_9O$.

(R)-2-((4-((5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)amino)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)-2-(4-methoxyphenyl)ethanol (16)

In a 10 ml CEM MW vial, (R)-2-amino-2-(4-methoxyphenyl)ethanol (105 mg, 0.63 mmol, NetChem Inc.), N-(5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (160 mg, 0.52 mmol**, IIId**), sodium carbonate (111 mg, 1.05 mmol), and DIPEA (0.091 ml, 0.52 mmol) were taken in butan-1-ol (5 ml). The RM was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.030g, 13.13 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.39 (s, 9H), 3.64 (d, *J*=3.8 Hz, 2H), 3.69 (s, 3H), 3.84 (s, 3H), 4.86 (br. s., 1H), 5.08 (br. s., 1H), 6.67 (br. s., 1H), 6.76-6.96 (m, 3H), 7.30 (d, *J*=8.5 Hz, 2H), 8.01 (br. s., 1H), 10.04 (br. s., 1H), 12.56 (br. s., 1H); HRMS: *m/z* (ES+); calculated, 437.23352, found, 437.24056 (MH^+) for $C_{22}H_{28}N_8O_2$.

(S)-2-((4-((5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)amino)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)-2-(3-fluorophenyl)ethanol (17)

In a 10 ml CEM MW vial, (S)-2-amino-2-(3-fluorophenyl)ethanol (97 mg, 0.63 mmol, NetChem Inc.), N-(5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (160 mg, 0.52 mmol, **IIId**), sodium carbonate (111 mg, 1.05 mmol), and DIPEA (0.091 ml, 0.52 mmol) were taken in butan-1-ol (5 ml). The RM was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under

vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.050g, 22.51 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.38 (s, 9H), 3.70 (t, *J*=5.8 Hz, 2H), 3.79 (s, 3H), 3.84 (s, 3H), 5.02 (m, 2H), 6.34-6.88 (m, 2H), 7.00 (d, *J*=4.7 Hz, 2H), 7.82-8.29 (m, 2H), 9.86-10.43 (m, 1H), 12.61 (br. s., 1H); HRMS: *m/z* (ES+); calculated, 425.23130, found, 425.22125 (MH^+) for $C_{21}H_{25}FN_8O$

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-(2-methoxypyridin-4-yl)ethanol (18)

In a 10 ml CEM MW vial, (R)-2-amino-2-(2-methoxypyridin-4-yl)ethanol (88 mg, 0.52 mmol, NetChem Inc.), N-(5-tert-butyl-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (160 mg, 0.52 mmol, **IIId**), sodium carbonate (111 mg, 1.05 mmol) and DIPEA (0.091 ml, 0.52 mmol) were taken in butan-1-ol (5 ml). The RM was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.020 g, 8.74 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.38 (s, 9H), 3.70 (t, *J*=5.7 Hz, 2H), 3.79 (s, 3H), 3.84 (s, 3H), 5.02 (m, 2H), 6.34-6.88 (m, 2H), 7.00 (d, *J*=4.7 Hz, 2H), 7.82-8.29 (m, 2H), 9.86-10.43 (m, 1H), 12.61 (br. s., 1H); HRMS: *m/z* (ES+); calculated, 438.22877, found, 438.2358 (MH^+) for $C_{21}H_{27}N_9O_2$.

(R)-2-((4-((5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)amino)-1H-pyrazolo[3,4-d]pyrimidin-6-yl)amino)-2-(3-chloropyridin-4-yl)ethanol (19)

In a 10 ml CEM MW vial, (R)-2-amino-2-(3-chloropyridin-4-yl)ethanol (108 mg, 0.63 mmol, NetChem Inc.), N-(5-(tert-butyl)-1-methyl-1H-pyrazol-3-yl)-6-chloro-1H-pyrazolo[3,4-d]pyrimidin-4-amine (160 mg, 0.52 mmol, **IIId**), sodium carbonate (111 mg, 1.05 mmol), and DIPEA (0.091 ml, 0.52 mmol) were taken in butan-1-ol (5 ml). The RM was subjected to microwave irradiation at 170 °C, 300 W for 45 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.020g, 8.65 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.48-1.62 (s, 9H), 3.70-3.92 (m, 2H), 3.94 (s, 3H), 5.29 (br. s., 1H), 5.50 (br. s., 1H), 6.82 (br. s., 1H), 7.18 (d, *J*=7.91 Hz,1H), 7.63 (br. s., 1H), 8.12 (br. s., 1H), 8.54 (d, *J*=5.0 Hz, 1H), 8.62-8.69 (m, 1H), 10.18 (br. s., 1H), 12.26-12.89 (br. s, 1H); HRMS: *m/z* (ES+); calculated, 442.17923, found, 442.18688 (MH^+) for $C_{20}H_{24}ClN_9O$.

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl hydroxycarbamate (20)

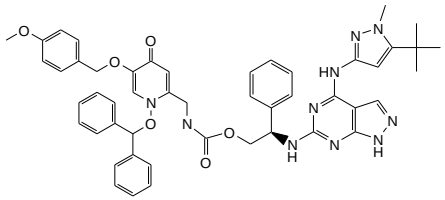
In a 50 ml round bottom flask, (R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (52 mg, 0.13 mmol, **10**) was dissolved in DCM. DIPEA (0.025 ml, 0.14 mmol) was added to it followed by addition of CDI (22.82 mg, 0.14 mmol). The RM was stirred at RT for 1 hr. The RM was diluted with DCM, the organic layer was washed with water (3X 10 ml). The combined organic layers were dried over sodium sulfate and concentrated under vacuum to obtain crude solid, which was taken in pyridine (5ml) and hydroxylamine hydrochloride (26.7 mg, 0.38 mmol) was added to it in one portion. The resulting reaction mixture was stirred at RT for 3 h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.020 g, 33.6 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.40 (s, 9 H) 3.84 (s, 3 H) 4.23 (br. s., 1 H) 4.29 (d, *J*=6.0 Hz, 1 H) 5.41 (s, 1 H) 7.24 (d, *J*=7.5 Hz, 2 H) 7.32 (br. s., 2 H) 7.44 (d, *J*=8.0 Hz, 2 H) 8.69 (br. s., 1 H) 9.64 (s, 1 H) 12.59 (br. s., 1 H); HRMS: *m/z* (ES+); calculated, 466.22369, found, 466.22367 (MH^+) for $C_{22}H_{27}N_9O_3$.

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl 2-hydroxyethylcarbamate (21)

In a 50 ml round-bottomed flask, (R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (50.0 mg, 0.12 mmol, **10**), TEA (0.051 ml, 0.37 mmol) and CDI (39.9 mg, 0.25 mmol) were taken in DCM (5 ml). The resulting reaction mixture was stirred at RT for 1 h. Then, ethanolamine (0.030 ml, 0.49 mmol) was added to reaction mixture and stirring was at RT for 30 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The reaction mixture was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.035 g, 57.7 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.40 (s, 9 H) 2.95 - 3.08 (m, 2 H) 3.84 (s, 3 H) 4.14 - 4.34 (m, 2 H) 4.52 - 4.66 (m, 1 H) 5.20 - 5.54 (m, 1 H) 6.46 - 6.81 (br. s, 1 H) 7.16 - 7.37 (m, 5 H) 7.39 - 7.50 (m, 2 H) 7.82 - 8.14 (br. s, 1 H) 9.84 - 10.24 (br. s, 1 H) 12.42 - 12.73 (br. s, 1 H); HRMS: *m/z* (ES+); calculated, 494.25499, found, 494.26162 (MH^+) for $C_{24}H_{31}N_9O_3$.

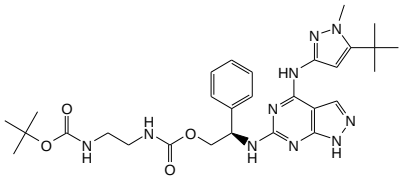
Intermediate Va

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl(1-(benzhydryloxy)-5-(4-methoxybenzyloxy)-4-oxo-1,4-dihydropyridin-2-yl)methylcarbamate



In a 50 ml round-bottomed flask, (R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (147 mg, 0.36 mmol, **compound 10)**, TEA (0.151 ml, 1.08 mmol) and CDI (117 mg, 0.72 mmol) were taken in DCM (10 ml). The resulting reaction mixture was stirred at RT for 1 hr followed by addition of 2-(aminomethyl)-1-(benzhydryloxy)-5-(4-methoxybenzyloxy)pyridin-4(1H)-one (400 mg, 0.90 mmol, **WO 2013150296**). The reaction mixture stirring at RT was continued for 2 days. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was concentrated to dryness and purified by reverse phase HPLC (Gilson) to afford title product (0.046 g, 14.54 %).
MS (ES+), (M+H)+ = 875.75 for $C_{49}H_{50}N_{10}O_6$

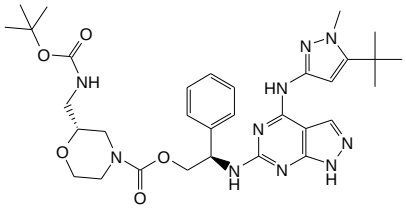
Intermediate Vb



In a 50 ml round-bottomed flask, (R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (100 mg, 0.25 mmol, **compound 10**), TEA (0.103 ml, 0.74 mmol) and CDI (48 mg, 0.30mmol) were taken in DCM (10 ml). The resulting reaction mixture was stirred at RT for 1 hr followed by addition of 2 tert-butyl 2-aminoethylcarbamate (64.0 mg, 0.40 mmol, Alfa Aesar**)** in DMF (2 ml). The reaction mixture stirring at RT was continued for 12 h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The RM was concentrated to dryness and directly used for next step. (0.120 g, 102 %).
MS (ES+), (M+H)+ = 593.53 for $C_{29}H_{40}N_{10}O_4$

Intermediate Vc

(R)-((R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl)2-((tert-butoxycarbonylamino)methyl)morpholine-4-carboxylate



In a 50 ml round-bottomed flask, (R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (100 mg, 0.25 mmol, **compound 10**), TEA (0.103 ml, 0.74 mmol) and CDI (80mg, 0.49 mmol) were taken DCM (5 ml). The resulting reaction mixture was stirred at RT for 1 h. Then, (S)-tert-butyl morpholin-2-ylmethylcarbamate (106 mg, 0.49 mmol, AZ Strategic Reagent Collection) was and stirred at RT for 12 h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. The reaction mixture was concentrated to dryness to afford title product (0.160 g, quantitative) directly used for next step.
MS (ES+), (M+H)+ = 649.47 for $C_{32}H_{44}N_{10}O_5$

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl 2-aminoethylcarbamate (22)

In a 25 ml round-bottomed flask**, Intermediate Vb** (120.0 mg, 0.20 mmol) was taken in DCM (4 ml). Trifluoro acetic acid was added to it (6 ml) and the resulting reaction mixture was stirred RT for 30 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The reaction mass was concentrated to dryness and purified by reverse phase HPLC (Gilson) to afford title product (0.055 g, 44.3 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.26-1.48 (s, 9H), 1.88 (s, 6H), 2.52-2.64 (m, 2H), 2.99 (d, *J*=6.0 Hz, 2H), 3.84 (s, 3H), 4.04-4.37 (m, 2H), 5.39 (br.s., 1H), 6.40-6.83 (m, 1H), 7.08-7.38 (m, 4H), 7.44 (d, *J*=7.3 Hz, 2H), 8.03 (br. s., 1H), 10.13 (br. s., 1H); HRMS: *m/z* (ES+); calculated, 493.27867, found, 493.27844 (MH^+) for $C_{24}H_{32}N_{10}O_2 \cdot 2$[Acetic acid].

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl(1,5-dihydroxy-4-oxo-1,4-dihydropyridin-2-yl)methylcarbamate (23)

In a 25 ml round-bottomed flask, (R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl(1-(benzhydryloxy)-5-(4-methoxybenzyloxy)-4-oxo-1,4-dihydropyridin-2-yl)methylcarbamate (46.0 mg, 0.05 mmol, **intermediate Va**) was taken in DCM (1 ml). Trifluoro acetic acid was added to it (2 ml, 25.96 mmol) and the resulting reaction mixture was stirred RT for 2 hours and at 45 °C for 6 hrs. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The reaction mass was concentrated to dryness and purified by reverse phase HPLC (Gilson) to afford title product (0.015 g, 44.0 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.39 (s, 9 H) 1.90 (s, 3 H) 3.84 (s, 3 H) 4.06 - 4.15 (m, 2 H) 4.19 - 4.32 (m, 2 H) 4.36 - 4.49 (m, 1 H) 5.38 - 5.58 (m, 1 H) 6.38 - 6.53 (m, 1 H) 6.58 - 6.80 (m, 1 H) 7.15 - 7.36 (m, 5

H) 7.40 - 7.51 (m, 2 H) 7.55 - 7.79 (m, 2 H) 7.92 - 8.17 (br. s, 1 H) 9.85 - 10.30 (br. s, 1 H); HRMS: *m/z* (ES+); calculated, 589.26371, found, 589.26295 (MH^+) for $C_{28}H_{32}N_{10}O_5$·[Acetic acid].

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl ((S)-3-methyl-2-oxoimidazolidin-4-yl)methylcarbamate (24)

In a 50 ml round-bottomed flask, (R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (60.0 mg, 0.15 mmol, **10**), TEA (0.062 mL, 0.44 mmol) and CDI (47.9 mg, 0.30 mmol) were taken in DCM (5 ml). The resulting reaction mixture was stirred at RT for 1 hr. Then, (R)-5-(aminomethyl)-1-methylimidazolidin-2-one (19.07 mg, 0.15 mmol, AZ Strategic Reagent Collection) was added to the reaction mixture and stirring was continued at RT for 12 h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The reaction was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.027 g, 32.6 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.40 (s, 9H),, 2.56 (s, 3H), 2.65-3.26 (m, 4H), 3.42 (br. s., 1H), 3.84 (s, 3H), 4.03-4.42 (m, 2H), 5.08-5.64 (m, 1H), 5.89-6.32 (m, 1H), 6.35-6.87 (m, 1H), 7.09-7.62 (m, 7H), 8.02 (br. s., 1H), 9.62-10.31 (m, 1H), 12.77 (br. s., 1H); HRMS: *m/z* (ES+); calculated, 562.2991, found, 562.2996 (MH^+) for $C_{27}H_{35}N_{11}O_3$.

(R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl 2-(3-oxopyrazolidin-1-yl)ethylcarbamate (25)

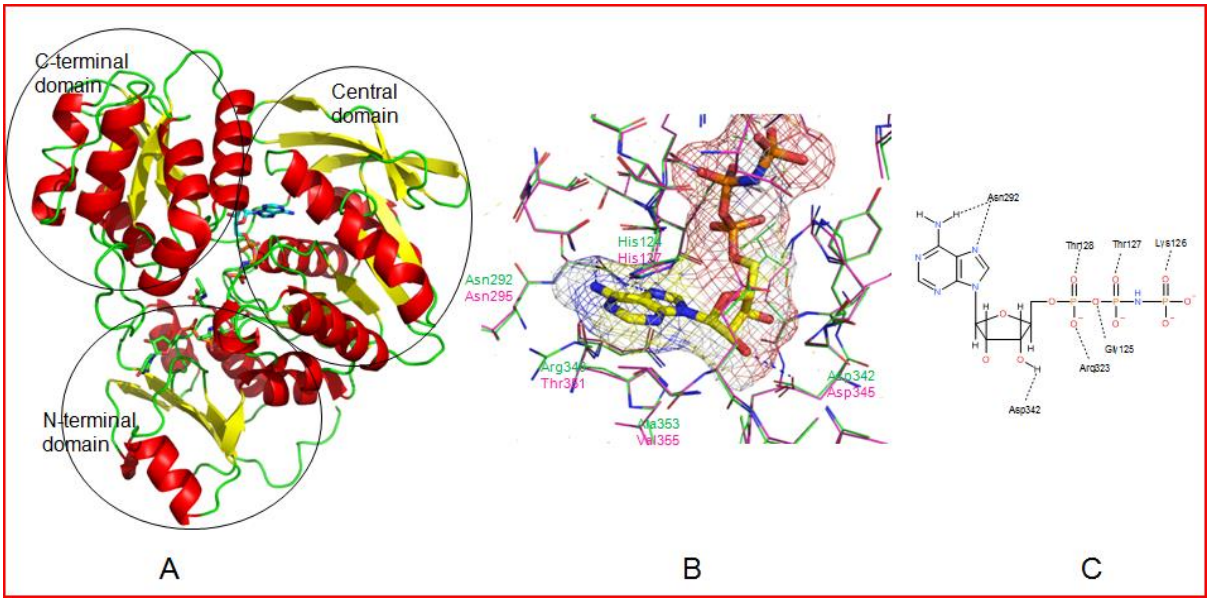
In a 50 ml round-bottomed flask, (R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethanol (75 mg, 0.18 mmol, **10**), TEA (0.077 ml, 0.55 mmol) and CDI (59.8 mg, 0.37 mmol) were taken in DCM (5 ml). The resulting reaction mixture was stirred at RT for 1 h. Then, 1-(2-aminoethyl)pyrazolidin-3-one (47.7 mg, 0.37 mmol, AZ Strategic Reagent Collection) was added to the reaction mixture and the stirring was continued at RT for 12 h. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product.The reaction mixture was filtered and washed with methanol. The combined organic layers were concentrated under vacuum to obtain crude solid which was dissolved in DMSO and purified by reverse phase HPLC (Gilson) to afford title product (0.025 g, 24.12 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.40 (s, 9 H) 2.22 - 2.37 (m, 2 H) 2.56 - 2.67 (m, 2 H) 2.98 - 3.21 (m, 4 H) 3.92 (s, 3 H) 4.09 - 4.32 (m, 2 H) 5.25 - 5.57 (m, 1 H) 6.44 - 6.80 (m, 1 H) 7.06 - 7.38 (m, 5 H) 7.39 - 7.49 (m, 2 H) 7.88 - 8.16 (br. s, 1 H) 9.40 (s, 1 H) 9.92 - 10.27 (br. s, 1 H) 12.45 - 12.73 (br. s, 1 H); HRMS: *m/z* (ES+); calculated, 562.29243, found, 562.29241(MH^+) for $C_{27}H_{35}N_{11}O_3$.

(R)-((R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl) 2-(aminomethyl)morpholine-4-carboxylate (26)

In a 25 ml round-bottomed flask, (R)-((R)-2-(4-(5-tert-butyl-1-methyl-1H-pyrazol-3-ylamino)-1H-pyrazolo[3,4-d]pyrimidin-6-ylamino)-2-phenylethyl)2-((tert-butoxycarbonylamino)methyl)morpholine-4-carboxylate (130 mg, 0.20 mmol, **intermediate Vc**) was taken in 4N HCl in Dioxane (3 ml, 98.74 mmol). The resulting reaction mixture was stirred 55 °C for 30 min. The progress of the reaction was monitored by LCMS and the profile showed the formation of required product. The reaction mass was neutralised with NaHCO3 solution, and extracted using 5% Methanol in DCM (3X 10 ml). The combined organic fractions were concentrated to dryness and purified by reverse phase HPLC (Gilson) to afford title product (0.027g, 24.56 %). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.16 - 1.33 (m, 1 H) 1.40 (s, 9 H) 2.07 - 2.43 (m, 1 H) 2.61 - 2.88 (m, 2 H) 2.99 - 3.23 (m, 2 H) 3.44 - 3.77 (m, 3 H) 3.84 (s, 3 H) 3.97 - 4.54 (m, 3 H) 5.34 - 5.69 (m, 1 H) 6.50 - 6.78 (m, 1 H) 7.19 - 7.28 (m, 1 H) 7.30 - 7.39 (m, 2 H) 7.42 - 7.52 (m, 2 H) 7.86 - 8.12 (br. s, 1 H) 9.93 - 10.30 (br. s, 1 H) 12.49 - 12.73 (br. s., 1 H); HRMS: *m/z* (ES+); calculated, 549.29719, found, = 549.30526 (MH^+) for $C_{27}H_{36}N_{10}O_{3.}$

S3 – Molecular Modelling

The homology model for *P. aeruginosa (Pae)* MurC was developed using SWISS MODEL (Arnold K., Bordoli L., Kopp J., and Schwede T. (**2006**). The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. *Bioinformatics*, 22,195-201). The complex structure reported for *H. influenzae* (*Hin*) (pdb ID: 1P3D) was used as a template. The QMEAN4 and GMQE scores are -1.65 and 0.82, respectively, suggesting that the model is fairly accurate. The model was further validated using Ramachandran plot (> 95 % of the residues are in the allowed regions of Φ and Ψ). The protein preparation wizard from Schrodinger Suite of programs was used for optimize the H-bond interaction followed by a constrained minimization. The resulting structure was used for docking.



FigureS3-1: N-terminal, central and C-terminal domains of *Pae* MurC from homology model. B. Active site overlay of *Hin* MurC (pdb ID: 1P3D) and *Pae* MurC (model); C. The key interactions with ANP are depicted in the 2D-interaction diagram.
Primary sequence alignment of MurC from bacterial pathogens (pink region is ATP binding domain, yellow region is UDP binding domain).

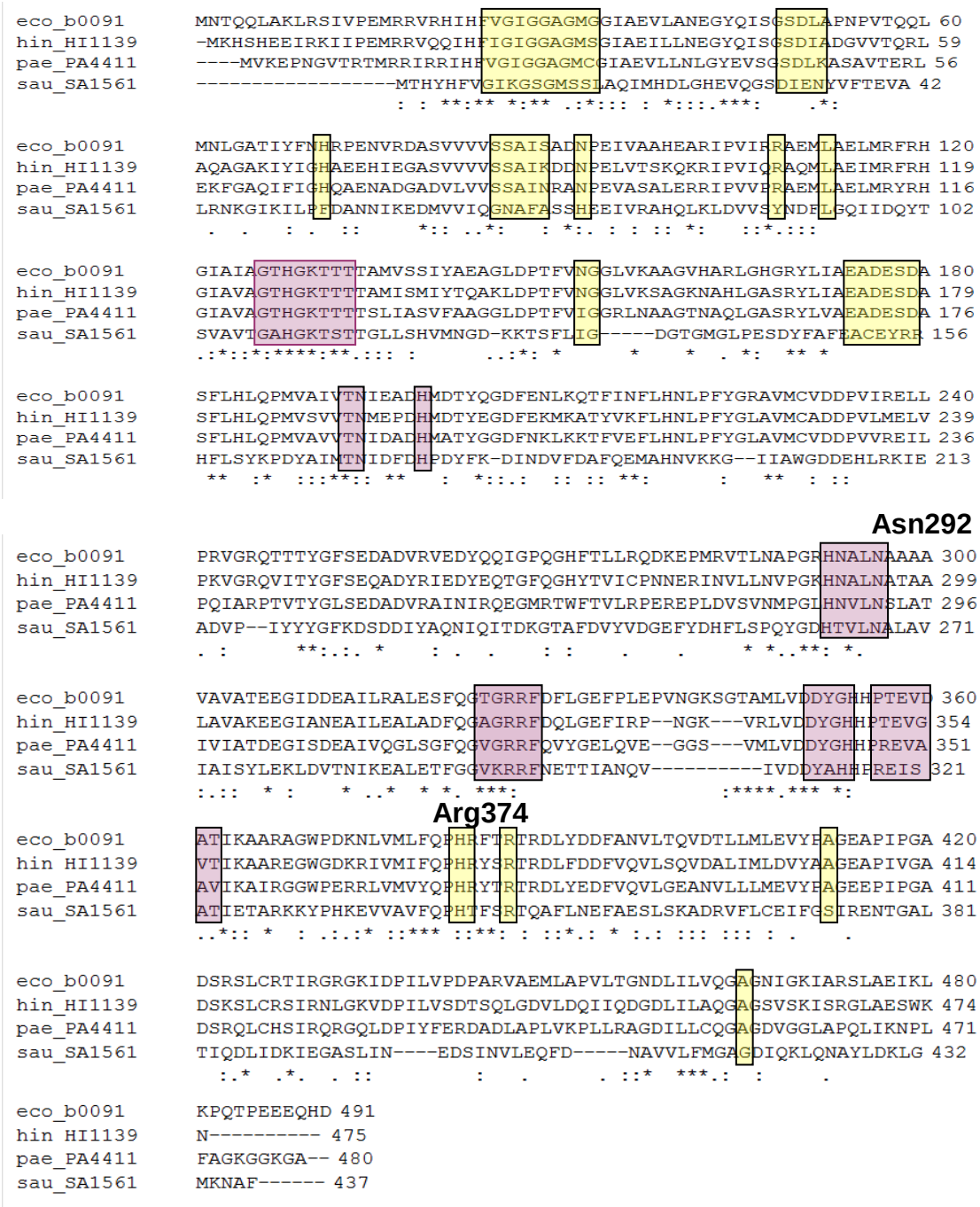


Figure S3-2: Primary sequence mapping of MurC from *E. coli* (eco_b0091), *H. influenza* (hin_HI1139), *P. aeruginosa* (pae_PA4411) and *S. aureus* (sau_SA1561). The residues highlighted in pink are from ATP binding site (5 Å radii) and the yellow highlighted residues are from UNP binding site (5 Å radii).

Table S3-1: Comparison of active site residues of MurC from bacterial pathogens.

Pae MurC	*Hin* MurC (1P3D)	*Eco* MurC	*Kpn*[a] MurC	Interactions / site feature
ASN292	ASN295	ASN296	ASN296	H-bonds with NH2 of ATP/ANP
HIS288	HIS291	HIS292	HIS292	pi-pi/cH-pi interaction with ring
ALA352	VAL355	ALA361	ALA361	
ARG348	THR351	THR357	THR357	
VAL350	THR356	THR362	THR362	
ASP342	ASP345	ASP351	ASP351	acceptor, ANP
ARG323	ARG326	ARG327	ARG327	acceptor ANP
TYR343	TYR346	TYR352	TYR352	donor, ANP
ARG374	ARG377	ARG383	ARG383	donor, acceptor ANP
ARG377	ARG380	ARG386	ARG386	acceptor ANP
THR128	THR131	THR132	THR132	donor, donor ANP
GLY125	GLY128	GLY129	GLY129	donor ANP
HIS124	HIS127	HIS128	HIS128	pi-pi, ANP
THR127	THR130	THR131	THR131	donor ANP
LYS126	LYS129	LYS130	LYS130	donor ANP
ALA26	ALA29	ALA30	ALA30	donor, UMA
CYS39	SER32	GLY33	GLY33	
MET28	MSE31	MSE32	MET32	
LYS48	ALA51	ALA52	ALA52	
LEU47	ILE50	LEU51	LEU51	donor, UMA
ASP46	ASP49	ASP50	ASP50	acceptor, UMA
ASN85	LYS88	SER89	SER89	

a. Klebsiella pneumoniae

S4: Antibacterial activity of compounds against wild-type *P. aeruginosa* (*Pae*) and *E. coli* (*Eco*) strains

Table S4-1: MIC in wild-type *Eco* and *Pae* strains

Compound #	MIC *Eco* (μM)	MIC *Pae* (μM)
6	>200	>200
7	>60	>60
8	>200	>200
9	>60	>60
10	>200	>200
11	>200	>200
12	>200	>200
13	>200	>200
14	>200	>200
15	>200	ND
16	>200	>200
17	>200	ND
18	>200	>200
19	>200	>200
20	>200	>200
21	>200	>200
22	>200	>200
23	>200	>200
24	>200	>200
25	>200	>200
26	200	>200

ND=Not done

S5: IC_{50} correlation between *Pae* and *Eco* MurC

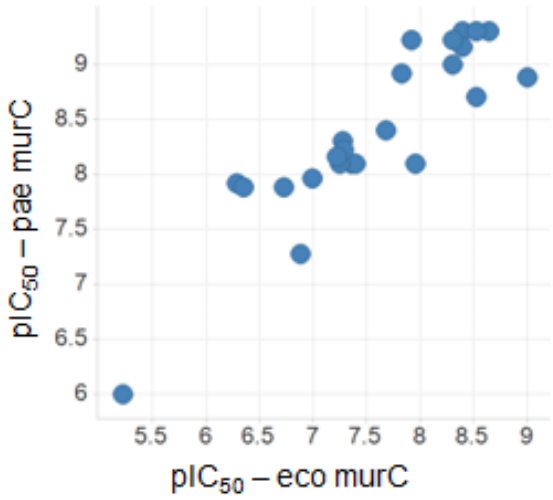


Figure S5-1: Correlation between enzymatic activity of *Pae* MurC and *Eco* MurC (IC_{50} values are given in log scale)

S6: Safety and DMPK profile

Table S6-1: Safety and DMPK profile of representative leads

No	A549_MMIC (µM)	Solubility (µM)	Hu_PPB (% free)	hERG IC_{50} (µM)	LogD (µM)
6	23.5	20	1.8	>33	>4.5
10	60	52	8.3	>33	3.3
12	>100	145	30.7	>33	2.6
14	>100	586	18	>33	2.4
15	>100	775	43	>33	2.3
19	>100	255	10.8	>33	2.9
22	>100	606	12.4	>33	1.3
26	>100	368	7.9	>33	2.0

A549_MMIC - Cytotoxicity from Hu A549 cell line: The *in vitro* cytotoxicity of compounds were measured against A549 human lung carcinoma cells as described earlier (Eakins et al. *Antimicrob. Agents Chemother.* **2012**, *56,* 1240-1246). **LogD:** Octanol-water partition coefficient (Log *D*) was measured by shake-flask method. A 10 mM sodium phosphate buffer pH 7.4 was used as aqueous solution. A 10 mM compound solution was made in DMSO and subsequently DMSO was removed using GeneVac. A 435 µl of octanol was added using Tomtec, stirred for 5 minutes. Further mixing was done by inversion for 5 h at 25 ºC,

subsequently centrifuged for 30 minutes at 3000 rpm. LC/UV/APPI/MS quantitation of both aqueous and octanol layers was performed to calculate Log *D* value according to the following equation. LogD = *Log* (Octanol/Octanol inj volume/ Buffer/Buffer inj volume). The method has been validated for log *D* ranging from -2 to 5.0**. hERG assay:** Compounds were tested on voltage-gated ion channels using the medium-throughput electrophysiology IonWorks™ device as described previously (Schroeder *et. al. J. Biomol. Screen.* 2003*, 8*, 50-64).

S7: Precursor incorporation studies

Precursor incorporation studies were performed to identify the macromolecular biosynthesis the test compounds could be inhibiting. Briefly, *E. coli lysA*$^{-}$ *tolC*$^{-}$ [Wietjes, F. B., Pas, E., Taschner, P. E. M., Woldringh, C. L. (1985) Kinetics of Uptake and Incorporation of meso-Diaminopimelic Acid in Different Escherichia coli Strains *Journal Bacteriol. 164(1)*, 331-337] cells were exposed to different concentrations of the test compounds for 60 minutes and the level of incorporation of radio-labeled precursors was measured. ^{14}C-Acetic acid, ^{14}C-Leucine, ^{3}H-DAP, ^{3}H-Thymidine and ^{3}H-Uridine were used to assess the synthesis of fatty acids, protein, peptidoglycan, DNA and RNA respectively. Triclosan, erythromycin, ampicillin, ciprofloxacin and rifampicin were used as reference drugs respectively.

Table S7-1: IC_{50} for precursor incorporation in macromolecular synthesis assay

Inhibitor	Incorporation IC_{50} μM for				
	Protein	Cell wall	Fatty acid	RNA	DNA
	^{14}C-Leucine	^{3}H-DAP	^{14}C-Acetic acid	^{3}H-Uridine	^{3}H-Thymidine
Erythromycin	4.9	348.8	348.8	348.8	348.8
Ampicillin	732.7	157.4	732.7	732.7	732.7
Triclosan	1.7	0.3	0.003	0.1	0.5
Rifampicin	12.2	311.1	311.1	4.9	303.8
Ciprofloxacin	30.2	772.6	772.6	0.1	0.8
6	202.0	4.4	202.0	202.0	202.0
10	621.4	0.7	621.4	621.4	621.4

S8: MIC modulation in MurC overexpression strains:

E. coli and *P. aeruginosa* MurC overexpression strains in *E. coli tolC*$^{-}$ (*Eco*524) were generated by transforming the recombinant plasmids pBAN0470 and pBAN0468 carrying the respective *murC* genes in the pBAD/Myc-HisA vector. PCR amplicon of *E. coli murC* gene

amplified using forward primer: ACGTATCCATGGGA AATACACAACAATTGGC and reverse primer: TGTCATGTTGTTCTTCCTCCGGAGT was cloned at *Nco*I and *Hind*III-end filled pBAD/Myc-HisA vector. Similarly, the *P. aeruginosa murC* gene was amplified with forward primer: ATACATCCATGGGAGTGGTTAAAGAACCG and reverse primer: ATATAAGCTT TGCGCCCTTCCCTCC and cloned at *Nco*I-*Hind*III digested pBAD/Myc-HisA vector. The recombinant strains were grown in MH broth supplemented with 50 μg/ml ampicillin until A600 ~0.5, arabinose was added to a final concentration of 0.4% and incubcated at 37^0C for an additional 3 hours. An uninduced culture was maintained as control. These cultures were used for protein analysis by coomassie staining and also for MIC modulation studies with both reference and test compounds following the NLSI guidelines.

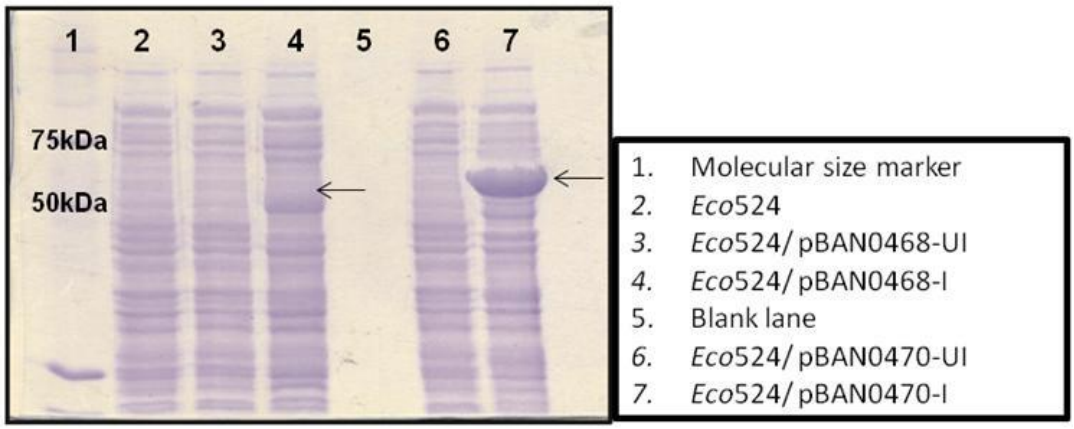
Table S8-1: MIC modulation studies in MurC over-expression strains

MIC (μM) in *Eco*524 and MurC over-expression strains in *Eco*524					
Inhibitor	*Eco*524	*Pae* MurC OE UI	*Pae* MurC OE I	*Eco* Murc OE UI	*Eco* MurC OE I
12	1.6	1.6	6.0	3.1	100
18	3.1	3.1	6.3	6.3	50
16	3.1	1.6	13.0	6.3	100
6	12.5	6.3	12.5	12.5	>100
20	25.0	25.0	100.0	25.0	>100
10	6.3	6.3	25.0	12.5	>100
Linezolid	3.1	3.1	3.1	3.1	3.1
Erythromycin	1.6	3.2	1.6	1.6	1.6
Meropenem	0.03	0.06	0.03	0.03	0.03
Ofloxacin	0.02	0.04	0.04	0.04	0.04
Novobiocin	3.9	3.9	1.9	3.9	3.9

OE: Overexprssion; I: Induced with 0.4% arabinose for 3 hours at 37 ^{0}C; UI: Uninduced

Figure S8-1:

Protein expression analysis of the MurC overexpression strains



S9: Generation and characterization of spontaneous resistant mutants

Spontaneous resistant mutants were raised against **6** and **10** by plating about 10^{10} cells of *P. aeruginosa546* strains on MH-agar plates supplemented with either 4X or 8X or 16X the MIC of these compounds. The isolated colonies were grown in plain MH broth and used to assess the level of resistance for the mentioned compounds in a tubridometric MIC assay. A number of reference inhibitors were also tested in parallel to ascertain the specificity. Genomic DNA was isolated from those colonies which showed specific up-shift in MIC for the test compounds only. *murC, murD, murE* and *murF* genes were PCR amplified using gene specific primers as listed below. All the amplicons were sequenced to identify the mutation responsible for resistance phenotype.

Primer name	Primer sequence (5’ ----- 3’)
PMrcSqF	CTACCCGGACGGTGGTCGATG
PMrcSqR:	GATCCTGGGTGCCGTTCAGCAG
PMrDSqF:	CACCGTGATCCTGGTGCTGATC
PMrDSqR:	GATGCCGTGCCGGCTCAACAG
PMrESqF:	CCTGCCGACGGCCACCGAACAG

PMrESqR:	CGGCGTCCTCGCCGATCAGG
PMrFSqF:	CGCCATCCGTTCTCCGACATCG
PMrFSqR:	GAGAATGCCGCGCAGGGTCAGG

S10: Colistin exposure to determine the MIC in wild-type *E. coli* K12 strain.

Wild-type *E. coli* K12 cultures grown in MH broth till A_{600} ~ 0.1 was exposed to 0.25 μg/ml of colistin for 30 minutes at 37 ^{o}C. The cells were harvested, washed once with fresh medium and resuspended to get 10^5 CFU / ml. MIC with test and reference inhibitors was set up using this culture. Concentration of the inhibitor giving 80% growth inhibition was taken as the MIC. Growth / growth inhibition was monitored by measuring A_{600} using Spectramax.

S11: MurC protein purification and assays to determine IC_{50}

E. coli murC gene was amplified from *E. coli* MG1655 genomic DNA using polymerase chain reaction (PCR) according to published procedures (Shapiro et. al). Amplification was performed using High Fidelity PCR Master (Roche Applied Science) using the following primers, synthesized with Nde I and Sal I restriction sites (EuroFins MWG Operon):
5'-GATCTGCATATGAATACACAACAATTGGC-3′
5′-CAGTACGTCGACTCAGTCATGTTGTTCTTC-3′
The purified PCR product (QuickStep 2 PCR Purification Kit) was digested with the restriction enzymes Nde I and Sal I (Roche Applied Science) and cloned into the appropriate sites of the expression vector pET-30a (Novagen). DNA sequence of the cloned *murC* gene was confirmed by sequencing.
The *E. coli murC* gene-containing plasmid was transformed into *E. coli* BL21 Star (DE3) pLysS cells, plated onto LB agar with 100 μg/ml ampicillin, and grown overnight at 37 °C. A starter culture grown overnight from a single colony was used to inoculate large volume of LB (with 100 μg/ml ampicillin) to get A_{600} ~0.1. This culture was grown at 30 °C till it reached A_{600} ~0.6, Temperature was lowered to 23 °C, and expression of the *murC* gene was induced with 0.5 mM IPTG overnight. Cells were harvested at a final A_{600} ~5 by centrifugation at 10500 g for 15 min.
MurC protein was purified as described previously (Marmor et al). Cell pellets were washed with 20 mM Tris-HCl, pH 7.5, and centrifuged again (5000 g, 10 min) at 4 °C. Cell paste was stored at −20 °C and protein expression checked by SDS−PAGE. The frozen cell paste was re-suspended in 100 ml of lysis buffer [20 mM Tris-HCl, pH 7.5, 2.5 mM DTT, 1 mM EDTA, 1 mM PMSF, and 2 protease inhibitor cocktail tablets (Roche Molecular Biochemical)], and

subjected to French press (2 × 18000 psi). The resulting effluent was centrifuged at 30000 rpm for 30 min at 4 °C, and the supernatant was loaded at a flow rate of 1 ml/min onto a Q-Sepharose HP (HR10/10) column (Pharmacia) pre-equilibrated with buffer A (20 mM Tris-HCl, pH 7.5, 1 mM EDTA, and 2.5 mM DTT). After washing the column with buffer A, the protein was eluted by a linear gradient from 0 to 1 M NaCl in buffer A. Fractions containing active protein were pooled, and $(NH_4)_2SO_4$ was added to give a final concentration of 1 M and loaded onto a phenyl-Sepharose HP (HR10/10) column (Pharmacia) pre-equilibrated with 1 M $(NH_4)_2SO_4$ in buffer A at a flow rate of 1 ml/min. After washing with 1 M $(NH_4)_2SO_4$ in buffer A, the protein was eluted using a linear gradient from 1 to 0 M $(NH_4)_2SO_4$ in buffer A.

Fractions containing active protein were pooled and dialyzed (1:100) at 4 °C for 6 h against buffer B (20 mM Tris-HCl, pH 7.5, 2.5 mM DTT, 10 mM $MgCl_2$, 150 mM NaCl, and 500 µM ATP), followed by an overnight dialysis (1:50) against buffer B containing 20% glycerol. The protein was characterized by SDS−PAGE analysis and analytical LC-MS and judged to be at >95% purity.

The *P. aeruginosa murC* gene was cloned, expressed and purified following a similar protocol. Primers used for amplification of *P. aeruginosa* PAO1 *murC* were

PamurC_expfor 5'- ACGTCATATGGTTAAAGAACCGAATGGCGTC-3'

PamurC_exprev 5'- ACGTGTCGACTCATGCGCCCTTCCCTCCCTTGCC

The reactions were carried out in a 25 µl volume in 25 mM Tris-HCl pH 8.0, 10 mM ammonium sulphate, 1.25 mM DTT, 0.002 % Brij-35, 10 mM $MgCl_2$, 40 µM UNAM, 100 µM ATP, 40 µM L-alanine and 15 nM enzyme in 384- well microtitre plate (Corning # 3702) at 25 °C for 50 minutes. Inorganic phosphate formed by the hydrolysis of ATP was detected by adding 25 µl of malachite green reagent prepared based on the protocol published by Baykov et al. An effective and stable malachite green formulation devised by us comprised of reagent A (0.12 % (W/V malachite green reagent in 6 N H_2SO_4), reagent B (7.5 % ammonium molybdate in water), reagent C (11 % (W/V) Tween-20) and water mixed in 10: 2.5 : 1 : 40.5 proportion on the day of the assay. A_{630} was measured after 30 minutes of incubation at 25 °C in spectramax plus (Molecular Devices). Percentage inhibition was calculated based on the A_{630} and fitted to four parameter Hill's equation to determine the IC_{50} of the compounds.

The reactions were carried out in a 50 µl volume in 50 mM Tris-HCl pH 8.0, 20 mM ammonium sulphate, 2.5 mM DTT, 0.002 % Brij-35, 1 mM $MgCl_2$, 18 µM UNAM, 38 µM ATP, 28 µM L-alanine, 2 µg/ml Poly uridine nucleic acid, 0.1 µM Polynucleotide Phosphorylase, 0.25X ribogreen and 3 nM enzyme in 384- well microtitre plate (Corning # 3573) at 25 °C for 60 minutes. Fluorescence was measured in Tecan Saffire II with excitation wavelenth at 485 nm and emission at 535 nm. Percentage inhibition was calculated based on ∆ Fluorescence (Fluorescence at 60 minutes - Fluorescence at 10 minutes) and fitted to a four parameter Hill's equation to determine the IC_{50} of the compounds.

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Intensity–Based Assay for UDP-NAcetylenolpyruvylglucosamine Reductase (MurB) with Nanomolar Product Detection. J. of Biomolecular Screening 17 327–338.

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- Baykov, A. A., Evtushenko, O. A., Avaeva, S. M. (1988). A malachite green procedure for orthophosphate determination and its use in alkaline phosphatase-based enzyme *immunoassay Anal. Biochem. 171(2)*, 266-270.

S12: Plasmids and strains used in this study

Strains / plasmids	**Genotype / description**	**Source**
*Eco*524	*E. coli tolC*$^{-}$	Lab strain
*Pae*546	*P. aeruginosa ΔmexABCDXY*	Lab strain
E. coli K12	Wild--type *E. coli*	Lab strain
pBAN0468	*Pae murC* gene cloned in pBAD/Myc-HisA vector	This study
pBAN0470	*Eco murC* gene cloned in pBAD/Myc-HisA vector	This study
10^{R}-P8N1	*Pae*546 resistant to compound 10	This study
10^{R}-P16N1	*Pae*546 resistant to compound 10	This study
6^{R}-P4N1	*Pae*546 resistant to compound 6	This study