Electronic Supplementary Information

Pd/Cu-catalyzed access to novel 3-(benzofuran-2-ylmethyl) substituted (pyrazolo / benzo)triazinone derivatives: their *in silico* / *in vitro* evaluation as inhibitors of chorismate mutase (CM)

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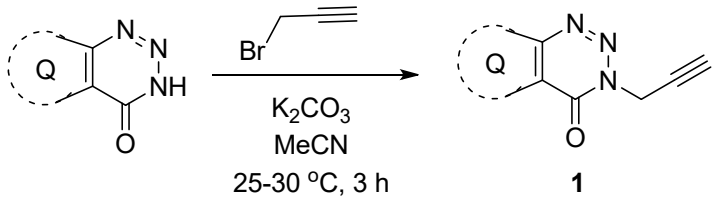
Table of contents

Experimental Section

Chemistry

General methods: Unless stated otherwise, reactions were performed under nitrogen atmosphere using oven dried glassware. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230-400 mesh) using distilled hexane and EtOAc. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 solution by using a 400 and 100 MHz spectrometer. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), td (triplet of doublet), t (triplet), q (quartet), and m (multiplet) as well as bs (broad singlet). Coupling constants (*J*) are given in hertz. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on Agilent 6430 series Triple Quard LC-MS / MS spectrometer. Chromatographic purity by HPLC (Agilent 1200 series ChemStation software) was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, diluent, detection wavelength, and retention times.

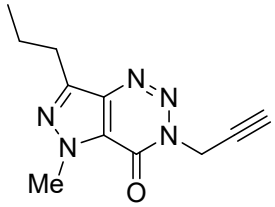
General procedure for the preparation of compound 1



(Q = benzene / substituted pyrazole ring)

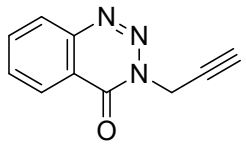
Propargyl bromide (1.2 mmol) was added to a solution of **1** (1.0 mmol) and K_2CO_3 (3.0 mmol) in CH_3CN (15 mL) under a nitrogen atmosphere. The mixture was stirred at ambient temperature (25-30 °C) for 5 h. After completion of the reaction (confirmed by TLC), the mixture was diluted with ice-water (60 mL) and extracted with EtOAc (3 x 15 mL). The organic layers were collected, combined, dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The residue was purified by column chromatography using 10% EtOAc in hexane as eluent to afford the title compound.

5-Methyl-3-(prop-2-yn-1-yl)-7-propyl-3*H*-pyrazolo[4,3-*d*][1,2,3]triazin-4(5*H*)-one (1a)



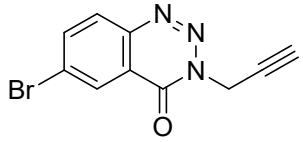
White solid (78% yield); mp 55-57 °C; R_f (20% EtOAc/*n*-hexane) 0.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.19 (d, *J* = 2.4 Hz, 2H, NCH_2), 4.29 (s, 3H, NCH_3), 3.01(t, *J* = 7.6 Hz, 2H, CH_2), 2.37 (t, *J* = 2.4 Hz, 1H, HC≡C), 1.93-1.82 (m, 2H, $MeCH_2$), 1.01 (t, *J* = 7.6 Hz, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 149.8 (C=O), 148.6, 136.1, 124.7, 77.0 (-C≡), 73.2 (HC≡), 38.8 (CH_2), 38.6 (NMe), 27.8 (CH_2), 22.3 (CH_2), 13.9 (Me); IR (KBr) ν_{max} 3238, 2120, 1697, 1531 cm^{-1}; MS (ES mass): *m*/*z* 232.2 (M+1, 100%).

3-(Prop-2-yn-1-yl)benzo[*d*][1,2,3]triazin-4(3*H*)-one (1b)



White solid (80% yield); mp 103-105 °C; R_f (20% EtOAc/*n*-hexane) 0.35; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.38 (dd, *J* = 8.0, 1.2 Hz, 1H, ArH), 8.18 (d, *J* = 8.4 Hz, 1H, ArH), 8.01-7.94 (m, 1H, ArH), 7.85-7.80 (m, 1H, ArH),5.23 (d, *J* = 2.8 Hz, 2H, NCH_2), 2.38 (t, *J* = 2.8 Hz, 1H, HC≡C); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 154.9(C=O), 144.3, 135.1, 132.7, 128.5, 125.2, 119.9, 76.9 (-C≡), 73.3 (HC≡), 39.4 (CH_2); MS (ES mass): *m*/*z* 186.20 (M+1, 100%).

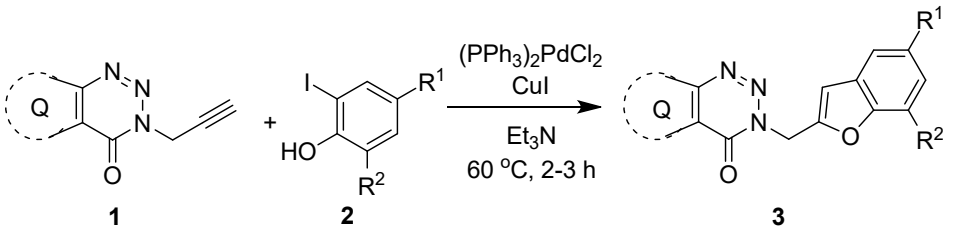
6-Bromo-3-(prop-2-yn-1-yl)benzo[*d*][1,2,3]triazin-4(3*H*)-one (1c)



White solid (75% yield); mp 92-94 °C; R_f (20% EtOAc/*n*-hexane) 0.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.12-7.95 (m, 2H, ArH), 8.51 (s, 1H, ArH), 5.21 (d, *J* = 2.8 Hz, 2H, NCH_2), 2.38 (t, *J* = 2.8 Hz, 1H, HC≡C); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 153.6 (C=O), 142.9, 138.5, 130.2, 128.0, 127.4, 121.2, 76.6 (-C≡), 73.5 (HC≡), 39.5 (CH_2); HPLC: 99.5%, column: X Bridge C-18 150*4.6 mm 5µm, mobile phase A: 5mM Ammonium Acetate in water, mobile phase B:

CH_3CN, (gradient) T/B% : 0/10, 23/90, 30/90, 31/90, 35/10; flow rate: 1 mL/min; Max plot, retention time 13.6 min; MS (ES mass): *m*/*z* 265.9 (M+2, 100%).

General procedure for the preparation of compound 3

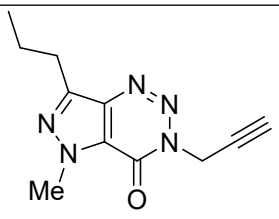
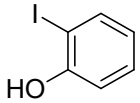
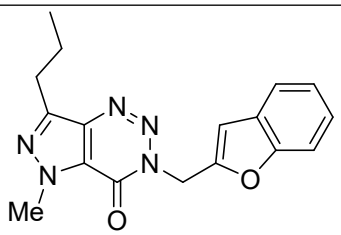


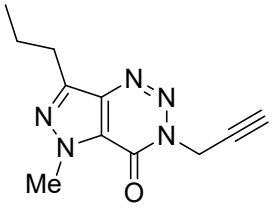
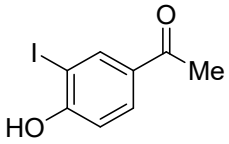
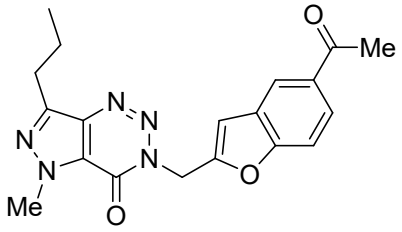
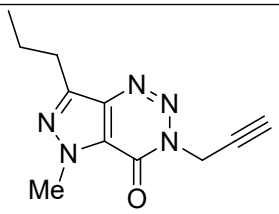
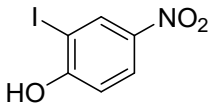
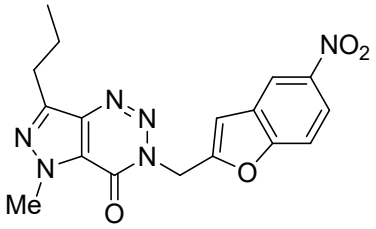
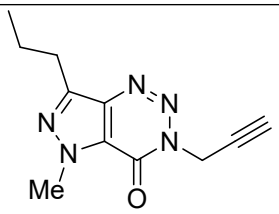
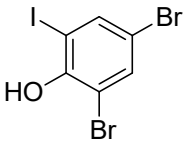
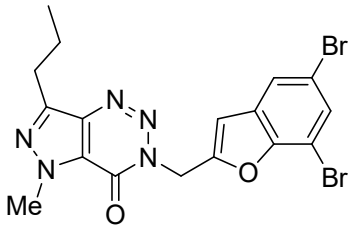
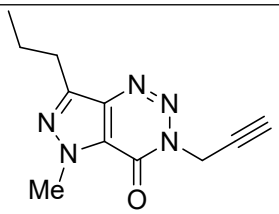
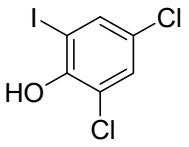
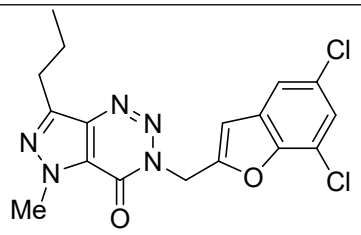
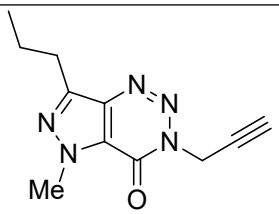
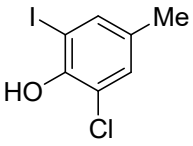
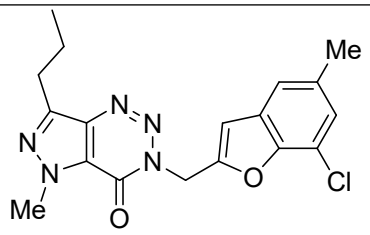
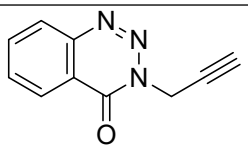
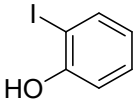
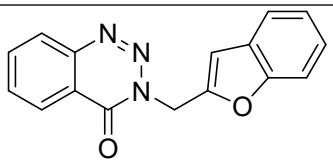
1 **2** **3**

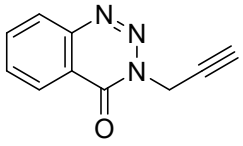
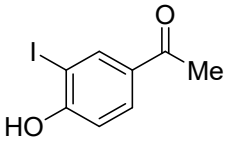
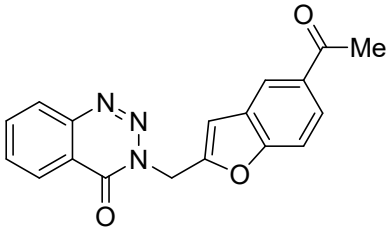
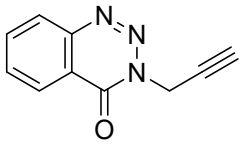
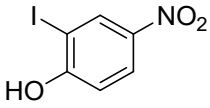
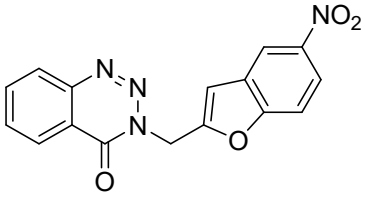
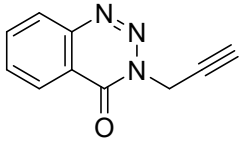
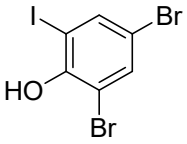
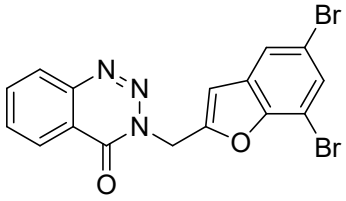
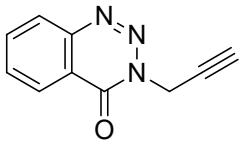
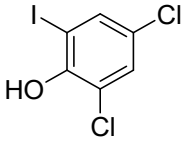
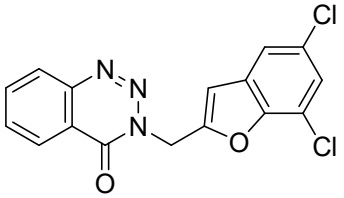
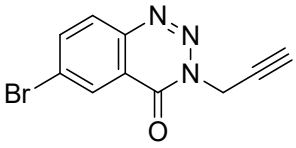
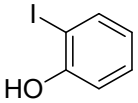
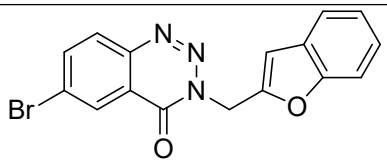
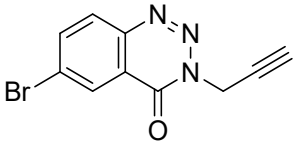
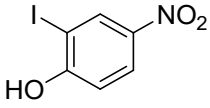
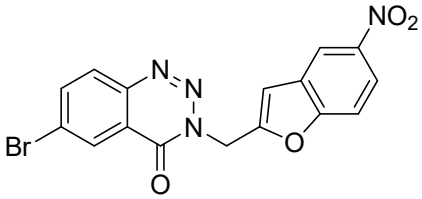
(Q = benzene / substituted pyrazole ring)

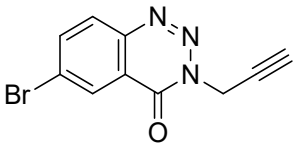
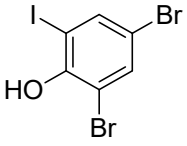
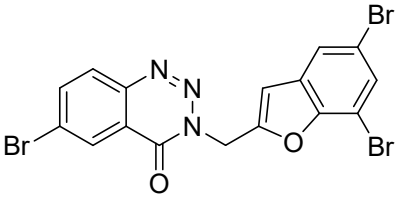
A mixture of compound **1** (1.0 mmol), *o*-iodophenol **2** (1.2 mmol), $(PPh_3)_2PdCl_2$ (5 mol%), CuI (5 mol%) in triethylamine (5 mL) was stirred at 60 °C for 2-3 h. After completion of the reaction (indicated by TLC) the reaction mixture was diluted with EtOAc (50 mL) and filtered through celite bed. The organic layer was collected, combined, washed with water (3 × 30 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under low vacuum. The crude residue was purified by column chromatography on silica gel using 10% EtOAc in hexane to afford the desired product. All the prepared compounds (**3a-n**) were characterized by MS, NMR spectra and their purity was determined by HPLC method.

Table S-1. Preparation of 3-(benzofuran-2-ylmethyl) substituted (pyrazolo / benzo)triazinone derivatives (**3**).

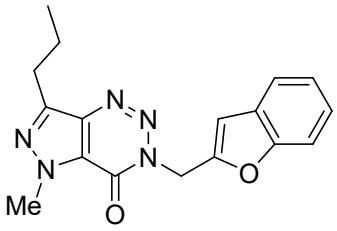
Entry	Alkyne (1)	*o*-iodophenol (2)	Product (3)
1	 (**1a**)	 (**2a**)	 **3a** (85%)

2	 (**1a**)	 (**2b**)	 **3b** (89%)
3	 (**1a**)	 (**2c**)	 **3c** (90%)
4	 (**1a**)	 (**2d**)	 **3d** (87%)
5	 (**1a**)	 (**2e**)	 **3e** (82%)
6	 (**1a**)	 (**2f**)	 **3f** (81%)
7	 (**1b**)		

		(**2a**)	**3g** (84%)
8	 (**1b**)	 (**2b**)	 **3h** (90%)
9	 (**1b**)	 (**2c**)	 **3i** (91%)
10	 (**1b**)	 (**2d**)	 **3j** (88%)
11	 (**1b**)	 (**2e**)	 **3k** (85%)
12	 (**1c**)	 (**2a**)	 **3l** (86%)
13	 (**1c**)	 (**2c**)	 **3m** (85%)

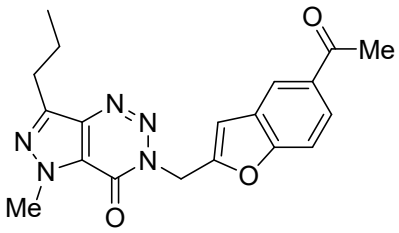
14	 (**1c**)	 (**2d**)	 **3n** (88%)

3-(Benzofuran-2-ylmethyl)-5-methyl-7-propyl-3*H*-pyrazolo[4,3-*d*][1,2,3]triazin-4(5*H*)-one (3a)



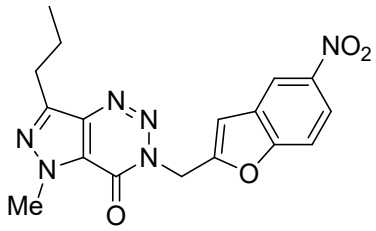
White solid (85% yield); mp: 60-62 °C; R_f = 0.76 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.60 (d, *J* = 7.2 Hz, 1H, ArH), 7.52 (d, *J* = 8.4 Hz, 1H, ArH), 7.30-7.20 (m, 2H, ArH), 6.94 (s, 1H, benzofuran (C-3) H), 5.74 (s, 2H, NCH_2), 4.18 (s, 3H, NCH_3), 2.93 (t, *J* = 7.6 Hz, 2H, CH_2), 1.86-1.73 (m, 2H, $\underline{CH_2}CH_3$), 0.94 (t, *J* = 7.2 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 154.2 (C=O), 152.2, 149.5, 146.8, 135.3, 127.8, 124.5, 124.5, 123.0, 121.2, 111.1, 105.9, 45.1 (NCH_2), 38.5 (NMe), 27.2 (CH_2), 21.6 ($\underline{CH_2}$Me), 13.7 ($CH_2\underline{Me}$); HPLC: 98.9%, Column: Cosmiscsil Aura ODS C-18 150*4.6 mm 5µm, mobile phase A: 5 mM Ammonium acetate in water, mobile phase B: MeCN, (gradient) T/B% : 0/10, 20/90, 28/90, 30/10, 35/10; flow rate: 1 mL/min, diluent: MeCN:water (80:20), UV: 210.0 nm, retention time 17.7 min; MS (ES mass): *m*/*z* 324.1 (M+1, 100%).

3-{(5-Acetylbenzofuran-2-yl)methyl)-5-methyl-7-propyl-3*H*-pyrazolo[4,3-*d*][1,2,3]triazin-4(5*H*)-one (3b)



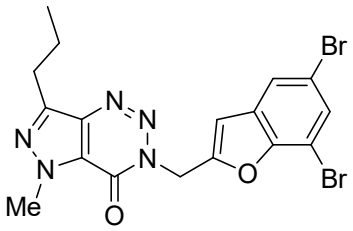
Pale yellow solid (89% yield); mp: 74-76 °C; R_f = 0.45 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.27 (d, *J* = 2.0 Hz, 1H, ArH), 7.90 (dd, *J* = 8.8, 2.0 Hz, 1H, ArH), 7.64 (d, *J* = 8.8 Hz, 1H, ArH), 7.08 (s, 1H, benzofuran (C-3) H), 5.76 (s, 2H, NCH_2), 4.16 (s, 3H, NCH_3), 2.92 (t, *J* = 7.6 Hz, 2H, CH_2), 2.61 (s, 3H, CO-$\underline{CH_3}$), 1.86-1.73 (m, 2H, $\underline{CH_2}CH_3$), 0.94 (t, *J* = 7.2 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.2 (C=O), 156.7, 153.8 (C=O), 149.5, 146.9, 135.3, 132.6, 127.6, 124.9, 124.5, 122.5, 111.2, 106.6, 45.0 (NCH_2), 38.4 (NMe), 27.2 (CH_2), 26.8 (CO-$\underline{Me}$), 21.6 ($\underline{CH_2}Me$), 13.6 ($CH_2\underline{Me}$); HPLC: 99.3%, Column: X-Bridge C-18 150*4.6 mm 5µm, mobile phase A: 5 mM Ammonium acetate in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:water (80:20), UV: 235.0 nm, retention time 14.9 min; MS (ES mass): *m*/*z* 366.1 (M+1, 100%).

5-Methyl-3-{(5-nitrobenzofuran-2-yl)methyl}-7-propyl-3*H*-pyrazolo[4,3-*d*][1,2,3]triazin-4(5*H*)-one (3c)



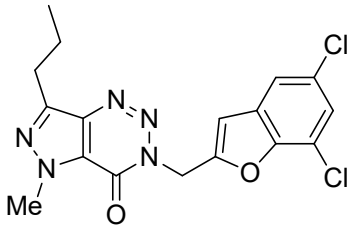
White solid (90% yield); mp: 98-100 °C; R_f = 0.64 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.57 (s, 1H, ArH), 8.16 (dd, *J* = 8.8, 2.4 Hz, 1H, ArH), 7.78 (dd, *J* = 8.8, 2.0 Hz, 1H, ArH), 7.18 (s, 1H, benzofuran (C-3) H), 5.80 (s, 2H, NCH_2), 4.17 (s, 3H, NCH_3), 2.93 (t, *J* = 7.6 Hz, 2H, CH_2), 1.86-1.73 (m, 2H, $\underline{CH_2}CH_3$), 0.94 (t, *J* = 7.2 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 157.1, 155.7 (C=O), 149.5, 146.9, 143.8, 135.3, 128.5, 124.5, 120.2, 117.7, 112.1, 106.9, 45.0 (NCH_2), 38.5 (NMe), 27.2 (CH_2), 21.6 ($\underline{CH_2}Me$), 13.6 ($CH_2\underline{Me}$); HPLC: 99.6%, Column: Eclipse XDB C-18 150*4.6 mm 5µm, mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in MeCN, (gradient) T/B% : 0/10, 5/10, 25/90, 30/90, 31/10, 35/10; flow rate: 1 mL/min, diluent: MeCN:water (80:20), UV: 240.0 nm, retention time 22.3 min; MS (ES mass): *m*/*z* 369.1 (M+1, 100%).

3-{(5,7-Dibromobenzofuran-2-yl)methyl)-5-methyl-7-propyl-3*H*-pyrazolo[4,3-*d*][1,2,3]triazin-4(5*H*)-one (3d)



White solid (87% yield); mp: 98-100 °C; R_f = 0.73 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.84 (d, *J* = 1.6 Hz, 1H, ArH), 7.72 (d, *J* = 1.6 Hz, 1H, ArH), 7.03 (s, 1H, benzofuran (C-3) H), 5.77 (d, *J* = 4.01 Hz, 2H, NCH_2), 4.18 (s, 3H, NCH_3), 2.92 (t, *J* = 7.6 Hz, 2H, CH_2), 1.86-1.73 (m, 2H, $\underline{CH_2}CH_3$), 0.94 (t, *J* = 7.2 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 154.8 (C=O), 150.4, 149.5, 146.9, 135.3, 130.8, 129.0, 124.5, 123.4, 115.6, 106.4, 104.1, 45.0 (NCH_2), 38.5 (NMe), 27.2 (CH_2), 21.6 ($\underline{CH_2}$Me), 13.6 ($CH_2\underline{Me}$); HPLC: 97.7%, Column: X-Bridge C-18 150*4.6 mm 5µm, mobile phase A: 5 mM Ammonium acetate in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:H_2O (80:20), UV: 220.0 nm, retention time 21.0 min; MS (ES mass): *m*/*z* 479.9 (M+, 50%), 481.9 (M+2, 100%), 483.9 (M+4, 50%).

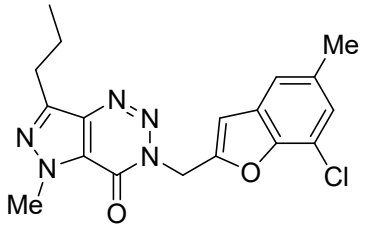
3-{(5,7-Dichlorobenzofuran-2-yl)methyl}-5-methyl-7-propyl-3*H*-pyrazolo[4,3-*d*][1,2,3]triazin-4(5*H*)-one (3e)



White solid (82% yield); mp: 90-92 °C; R_f = 0.73 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.68 (d, *J* = 2.0 Hz, 1H, ArH), 7.52 (d, *J* = 2.0 Hz, 1H, ArH), 7.02 (s, 1H, benzofuran (C-3) H), 5.77 (d, *J* = 4.01 Hz, 2H, NCH_2), 4.17 (s, 3H, NCH_3), 2.92 (t, *J* = 7.6 Hz, 2H, CH_2), 1.86-1.73 (m, 2H, $\underline{CH_2}CH_3$), 0.94 (t, *J* = 7.2 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 155.1 (C=O), 149.5, 148.6, 146.9, 135.3, 130.6, 127.8, 124.5, 124.0, 120.0, 116.1, 106.6, 45.0 (NCH_2), 38.5 (NMe), 27.2 (CH_2), 21.6 ($\underline{CH_2}$Me), 13.6 ($CH_2\underline{Me}$);

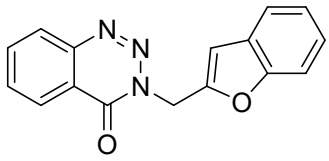
HPLC: 94.3%, Column: Eclipse Plus C-18 150*4.6 mm 5µm, mobile phase A: 10 mM Ammonium acetate in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:H_2O (10:90), UV: 210.0 nm, retention time 22.9 min; MS (ES mass): *m*/*z* 392.0 (M+1, 100%).

3-{(7-Chloro-5-methylbenzofuran-2-yl)methyl}-5-methyl-7-propyl-3*H*-pyrazolo[4,3-*d*][1,2,3]triazin-4(5*H*)-one (3f)



White solid (81% yield); mp: 73-75 °C; R_f = 0.76 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 7.35 (d, *J* = 0.8 Hz, 1H, ArH), 7.21 (d, *J* = 0.8 Hz, 1H, ArH), 6.95 (s, 1H, benzofuran (C-3) H), 5.74 (s, 2H, NCH_2), 4.18 (s, 3H, NCH_3), 2.93 (t, *J* = 7.6 Hz, 2H, CH_2), 2.34 (s, 3H, Ar-CH_3), 1.86-1.73 (m, 2H, $\underline{CH_2}CH_3$), 0.95 (t, *J* = 7.2 Hz, 3H, $CH_2\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 153.5 (C=O), 149.5, 148.1, 146.8, 135.3, 133.9, 129.7, 125.3, 124.5, 120.0, 114.5, 106.5, 45.0 (NCH_2), 38.4 (NMe), 27.2 (CH_2), 21.6 ($\underline{CH_2}$Me), 20.5 (Ar-Me), 13.6 ($CH_2\underline{Me}$); HPLC: 90.0%, Column: Eclipse Plus C-18 150*4.6 mm 5µm, mobile phase A: 10 mM Ammonium acetate in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:H_2O (10:90), UV: 210.0 nm, retention time 22.4 min; MS (ES mass): *m*/*z* 372.1.0 (M+1, 100%).

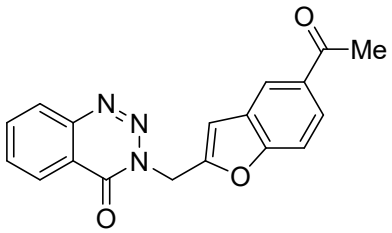
3-(Benzofuran-2-ylmethyl)benzo[*d*][1,2,3]triazin-4(3*H*)-one (3g)



Yellow solid (84% yield); mp: 100-102 °C; R_f = 0.77 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.27 (dd, *J* = 8.0, 1.2 Hz, 1H, ArH), 8.22 (d, *J* = 8.4 Hz, 1H, ArH), 8.14-8.07 (m, 1H, ArH), 7.96-7.92 (m, 1H, ArH), 7.63-7.58 (m, 1H, ArH), 7.53 (t, *J* = 7.6 Hz, 1H, ArH), 7.29-6.97 (m, 2H, ArH), 6.97 (s, 1H, benzofuran (C-3) H), 5.77 (s, 2H, NCH_2) ;

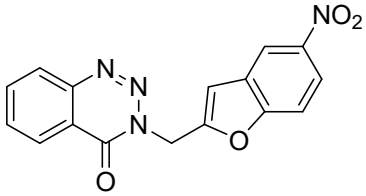
^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 154.5 (C=O), 154.3, 152.2, 143.6, 135.6, 133.2, 128.2, 127.8, 124.6, 124.5, 123.1, 121.2, 119.3, 111.1, 105.8, 46.0 (NCH_2); HPLC: 97.7%, Column: Eclipse XDB C-18 150*4.6 mm 5µm, mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in MeCN, (gradient) T/B% : 0/10, 5/10, 25/90, 30/90, 31/10, 35/10; flow rate: 1 mL/min, diluent: MeCN:water (80:20), UV: 210.0 nm, retention time 20.2 min; MS (ES mass): *m*/*z* 277.1 (M+1, 55%), 131.0 (M-146, 100%).

3-{(5-Acetylbenzofuran-2-yl)methyl}benzo[*d*][1,2,3]triazin-4(3*H*)-one (3h)



Pale yellow solid (90% yield); mp: 130-132 °C; R_f = 0.43 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.28-8.24 (m, 2H, ArH), 8.23 (d, *J* = 8.0 Hz, 1H, ArH), 8.13-8.08 (m, 1H, ArH), 7.97-7.92 (m, 1H, ArH), 7.90 (dd, *J* = 8.8, 2.0 Hz, 1H, ArH), 7.64 (d, *J* = 8.8 Hz, 1H, ArH), 7.12 (s, 1H, benzofuran (C-3) H), 5.80 (s, 2H, NCH_2), 2.60 (s, 3H, CO-$\underline{CH_3}$); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 197.2 (C=O), 156.7, 154.6 (C=O), 153.8, 143.6, 135.7, 133.2, 132.6, 128.2, 128.0, 124.9, 124.7, 122.5, 119.3, 111.3, 106.6, 46.0 (NCH_2), 26.8 (CO-$\underline{Me}$); HPLC: 99.8%, Column: Eclipse XDB C-18 150*4.6 mm 5 µm, mobile phase A: 0.1% TFA in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:water (20:80), UV: 235.0 nm, retention time 13.8 min; MS (ES mass): *m*/*z* 320.0 (M+1, 100%).

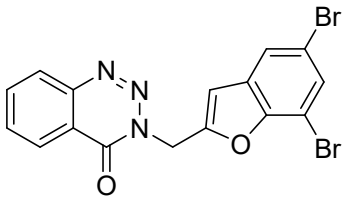
3-{(5-Nitrobenzofuran-2-yl)methyl}benzo[*d*][1,2,3]triazin-4(3*H*)-one (3i)



White solid (91% yield); mp: 160-162 °C; R_f = 0.51 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.58 (d, *J* = 2 Hz, 1H, ArH), 8.27 (d, *J* = 7.6 Hz, 1H, ArH), 8.24

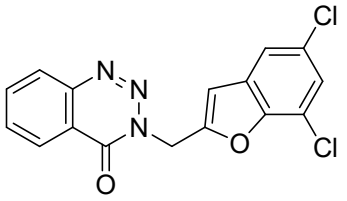
(d, *J* = 8.4 Hz, 1H, ArH), 8.17 (dd, *J* = 8.8, 2.0 Hz, 1H, ArH), 8.11 (t, *J* = 7.2 Hz, 1H, ArH), 7.96 (t, *J* = 7.6 Hz, 1H, ArH), 7.79 (d, *J* = 9.2 Hz, 1H, ArH), 7.22 (s, 1H, benzofuran (C-3) H), 5.85 (s, 2H, NCH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 157.2, 155.7, 154.6 (C=O), 143.8, 143.6, 135.7, 133.3, 128.5, 128.2, 124.7, 120.2, 119.4, 117.7, 112.1, 106.8, 45.9 (NCH_2); HPLC: 99.3%, Column: Eclipse Plus C-18 250*4.6 mm 5µm, mobile phase A: 5 mM Ammonium acetate in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:water (80:20), UV: 230.0 nm, retention time 14.7 min; MS (ES mass): *m*/*z* 323.1 (M+1, 50%), 176.1 (M-146, 100%).

3-{(5,7-Dibromobenzofuran-2-yl)methyl}benzo[*d*][1,2,3]triazin-4(3*H*)-one (3j)



White solid (88% yield); mp: 133-135 °C; R_f = 0.60 (20% EtOAc/*n*-hexane); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 8.28-8.22 (m, 2H, ArH), 8.11 (t, *J* = 7.6 Hz, 1H, ArH), 7.95 (t, *J* = 7.6 Hz, 1H, ArH), 7.83 (d, *J* = 1.6 Hz, 1H, ArH), 7.70 (d, *J* = 1.6 Hz, 1H, ArH), 7.07 (s, 1H, benzofuran (C-3) H), 5.81 (s, 2H, NCH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 154.7 (C=O), 154.5, 150.4, 143.6, 135.7, 133.2, 130.8, 129.0, 128.2, 124.6, 123.4, 119.3, 115.6, 106.4, 104.1, 45.9 (NCH_2); HPLC: 98.2%, Column: X-Bridge C-18 150*4.6 mm 5µm, mobile phase A: 5 mM Ammonium acetate in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:H_2O (80:20), UV: 220.0 nm, retention time 19.0 min; MS (ES mass): *m*/*z* 433.8 (M+, 50%), 435.8 (M+2, 100%), 437.8 (M+4, 50%).

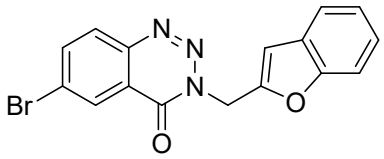
3-{(5,7-Dichlorobenzofuran-2-yl)methyl}benzo[*d*][1,2,3]triazin-4(3*H*)-one (3k)



White solid (85% yield); mp: 120-122 °C; R_f = 0.58 (20% EtOAc/*n*-hexane); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 8.30-8.22 (m, 2H, ArH), 8.15-8.09 (m, 1H, ArH), 7.99-7.92 (m,

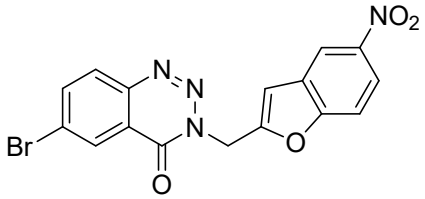
1H, ArH), 7.69 (d, *J* = 2.0 Hz, 1H, ArH), 7.53 (d, *J* = 2.0 Hz, 1H, ArH), 7.08 (s, 1H, benzofuran (C-3) H), 5.81 (s, 2H, NCH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 155.1 (C=O), 154.6, 148.6, 143.6, 135.7, 133.3, 130.7, 128.2, 127.8, 124.7, 124.0, 120.0, 119.4, 116.1, 106.5, 45.9 (NCH_2); HPLC: 93.9%, Column: Cosmicsil Aura ODS C-18 150*4.6 mm 5µm, mobile phase A: 0.05% TFA in water, mobile phase B: 0.05% TFA in MeCN, (gradient) T/B% : 0/10, 20/90, 28/90, 30/10, 35/10; flow rate: 1 mL/min, diluent: MeCN:water (80:20), UV: 215.0 nm, retention time 19.0 min; MS (ES mass): *m*/*z* 345.9 (M+, 50%), 198.9 (M-146, 100%).

3-(Benzofuran-2-ylmethyl)-6-bromobenzo[*d*][1,2,3]triazin-4(3*H*)-one (3l)



White solid (86% yield); mp: 94-96 °C; R_f = 0.79 (20% EtOAc/*n*-hexane); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 8.36 (d, *J* = 2.0 Hz, 1H, ArH), 8.26 (dd, *J* = 8.8, 2.0 Hz, 1H, ArH), 8.16 (d, *J* = 8.8 Hz, 1H, ArH), 7.60 (d, *J* = 7.2 Hz, 1H, ArH), 7.52 (d, *J* = 8.0 Hz, 1H, ArH), 7.30-7.20 (m, 2H, ArH), 6.98 (s, 1H, benzofuran (C-3) H), 5.77 (s, 2H, NCH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 154.3 (C=O), 153.4, 151.9, 142.5, 138.6, 130.4, 127.8, 127.0, 126.4, 124.5, 123.0, 121.2, 121.0, 111.1, 106.0, 46.2 (NCH_2); HPLC: 99.1%, Column: Eclipse XDB C-18 150*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:H_2O (80:20), UV: 210.0 nm, retention time 17.9 min; MS (ES mass): *m*/*z* 356.0 (M+1, 100%).

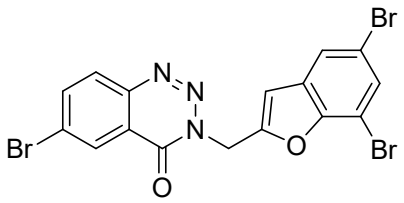
6-Bromo-3-{(5-nitrobenzofuran-2-yl)methyl}benzo[*d*][1,2,3]triazin-4(3*H*)-one (3m)



White solid (85% yield); mp: 146-148 °C; R_f = 0.67 (20% EtOAc/*n*-hexane); ^1H NMR (400 MHz, DMSO-d_6) δ ppm: 8.58 (d, *J* = 2.0 Hz, 1H, ArH), 8.37 (d, *J* = 2.0 Hz, 1H, ArH), 8.27 (dd, *J* = 9.2, 2.0 Hz, 1H, ArH), 8.18 (dd, *J* = 9.2, 2.0 Hz, 2H, ArH), 7.79 (d, *J* = 9.2 Hz, 1H, ArH), 7.23 (s, 1H, benzofuran (C-3) H), 5.83 (s, 2H, NCH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ

ppm: 157.2, 155.4 (C=O), 153.5, 143.8, 142.5, 138.6, 130.5, 128.5, 127.0, 126.5, 121.0, 120.3, 117.8, 112.1, 107.0, 46.1 (NCH_2); HPLC: 97.3%, Column: X-Bridge C-18 150*4.6 mm 5µm, mobile phase A: 0.1% TFA in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:water (80:20), UV: 240.0 nm, retention time 17.0 min; MS (ES mass): *m*/*z* 400.9 (M+, 98%), 402.9 (M+2, 100%).

6-Bromo-3-{(5,7-dibromobenzofuran-2-yl)methyl}benzo[*d*][1,2,3]triazin-4(3*H*)-one (3n)



White solid (88% yield); mp: 140-142 °C; R_f = 0.76 (20% EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm: 8.36 (s, 1H, ArH), 8.26 (d, *J* = 8.4 Hz, 1H, ArH), 8.17 (d, *J* = 8.4 Hz, 1H, ArH), 7.84 (s, 1H, ArH), 7.71 (s, 1H, ArH), 7.08 (s, 1H, benzofuran (C-3) H), 5.80 (s, 2H, NCH_2); ^{13}C NMR (100 MHz, DMSO-d_6) δ ppm: 154.5 (C=O), 153.4, 150.5, 142.5, 138.6, 130.8, 130.4, 129.0, 126.9, 126.5, 123.4, 121.0, 115.6, 106.6, 104.1, 46.1 (NCH_2); HPLC: 99.1%, Column: Eclipse XDB C-18 150*4.6 mm 5µm, mobile phase A: 10 mM Ammonium acetate in water, mobile phase B: MeCN, (gradient) T/B% : 0/5, 20/90, 30/90, 31/5, 35/5; flow rate: 1 mL/min, diluent: MeCN:water (80:20), UV: 210.0 nm, retention time 12.9 min; MS (ES mass): *m*/*z* 511.7 (M+, 30%), 513.7 (M+2, 100%), 515.7 (M+4, 100%), 517.7 (M+6, 30%).

Docking studies

All ligand structures were built in MarvinSketch.[1] Protein (2FP2) as well as all ligands were prepared (means optimization, charge calculation, deletion of co-crystal ligand, and addition of hydrogen etc.) using AtuDock tool.[2] All ligands were docked at interface site of *Mtb*CM (which is homodimer) using reliable open-source tool AutoDock Vina.[3] The grid map was made up of 20X20X20 points using AutoGrid with 0.54, 3.92, and 42.25 as center_X, center_Y, and center_Z respectively. To search all possible conformation of ligands thoroughly, exhaustiveness value of 20 were used, which made the process little more time consuming, but accurately identified the best pose of individual ligand.

To check the reproducibility of docking result, we have performed docking of each individual ligand at least ten times and maximum difference found ±0.2. The validation of docking protocol

was done by re-docking the co-crystal ligand (TSA) and calculating RMSD difference between co-crystal one and docked one in PyMOL,[4] maximum RMSD was 1.713 (<2) that indicated accuracy of our docking protocol.

Pharmacology

In vitro assay for CM inhibition

Enzyme and Reagents

Mycobacterium tuberculosis chorismate mutase (*Mtb*CM) gene was PCR amplified and cloned into expression vector pET22b. *Mtb*CM was purified from over expressed culture of BL21 (DE3) harboring pET22b/ *Mtb*CM by Ni-NTA affinity chromatography. The substrate chorismic acid was obtained from Sigma (SIGMA cat # 1701).

The CM enzymatic assay

Activity of chorismate mutase enzyme was based on the direct observation of conversion of chorismate to prephenate spectrophotometrically at OD_{274}. The reaction volume of the assay was maintained at 100 µl. The substrate chorismic acid (2 mM) was pre incubated at 37 ºC for 5 min in the buffer containing 50 mM Tris-HCl (pH 7.5), 0.5 mM EDTA, 0.1 mg/ml bovine serum albumin, and 10 mM β-Mercaptoethanol. The reaction was started by adding 180 pmol of CM enzyme to the pre-warmed chorismic acid solution. Inhibitory screening of the test compounds against CM activity was measured at 30 µM (10 nM to 30 µM for concentration dependent study) concentration of the effectors. The reaction was allowed to proceed at 37 °C and was terminated after 5 min with 100 µl of 1 N HCl and absorbance was read at 274 nm. Alternatively, the reaction was allowed to proceed for further 10 min and was then terminated with 180 µM of 2.5 N NaOH and absorbance was measured at 320 nm. A blank with no enzyme for every reaction was kept as a control to account for the non-enzymatic conversion of chorismate to prephenate. The % of enzyme inhibition caused by the test compound was calculated by the following formula:

% inhibition = 100 – residual activity of CM

Residual activity of CM = $[A_{274} \{S + (E' + C)\} - A_{274} (S + C)] / [A_{274} (S + E) – A_{274} (S)]$

S = absorbance of the substrate (chorismic acid) at 274 nm; E' = absorbance of the enzyme (CM) at 274 nm with compound; E = absorbance of the enzyme (CM) at 274 nm without compound; C

= test compound; A_{274} indicates absorbance at 274 nm (this is replaced by A_{320} for absorbance at 320 nm).

Computational ADME prediction

Software and methods

The ADME predictions were performed by using the SwissADME web-tool,[5] where the molecules were drawn using Marvin JS (version 16.4.18, 2016) and converted into SMILES by JChem Web Services (version 14.9.29, 2013). Then the 3D conformations were generated through the StringMolExport function. All descriptors and important molecular parameters of physicochemical properties were computed by OpenBable API (version 2.3.0, 2012). The predictive models were mostly generated by Quantitative Structure-Property Relationship (QSPR) methods along with some other robust models. As the SwissADME is a web-based tool, all these process are done in an automated manner.

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