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Electronic Supplementary information

Hydroxamates can distinguish between NAD^+ and ATP -dependent DNA ligases

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All the authors have equal contribution in this manuscript

Experimental

General Chemistry:

Commercially available reagent grade chemicals were used as received. All reactions were followed by TLC on E. Merck Kieselgel 60 F_{254}, with detection by UV light, spraying a 20% $KMnO_4$ aq solution. Column chromatography was performed on silica gel (100-200 mesh E. Merck). IR spectra were recorded as thin films or in KBr solution with a Perkin Elmer Spectrum RX-1 (4000-450 cm^{-1}) spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Brucker DRX -200 in $CDCl_3$ and $CDCl_3$+CCl_4. Chemical shift values are reported in ppm relative to TMS (tetramethylsilane) as internal reference, unless otherwise stated; s (singlet), d (doublet), m (multiplet); *J* in hertz. ESI mass spectra were performed using Quattro II (Micromass). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer.

Typical Experimental Procedure for the synthesis of alkylated hydroxamic acids (3a-3c, 6a-6c, 16a-16b, 17a-17c, 18a-18e):

To a magnetically stirred solution of 1-hydroxy napthoic acid (1.0 eq) or salicylic acid (1.0 eq.) or 2,3/2,4/2,5-dihydroxy benzoic acid (1.0 eq) in methanol concentrated sulphuric acid (20% mol) was added and mixture was refluxed for 1h to get the corresponding methyl ester (**1**, **4, 7-9)**. Synthesis of monoalkylated derivatives **(10a-10b, 11a-11c, 12a-12e)** of **7-9** were achieved by the chemoselective alkylation of hydroxy group present at 3/4/5 position of aromatic ring in compound **7-9** with different alkyl halides (1.2-1.6 eq) in acetone in presence of anhydrous K_2CO_3 (1.0 eq) and catalytic amount of tetrabutylammonium bromide (TBAB) (20 mol%). To a magnetically stirred solution of methyl esters (**1**, **4**, 1 eq.) and monoalkylated methyl esters (**10a-10b, 11a-11c, 12a-12e**, 1.0 eq) in dry THF (10.0 ml), potassium carbonate (1.0 eq) was added followed by addition of alkyl halides (1.2-1.6 eq.) and tetrabutyl ammonium bromide (20 mol%),

the reaction mixture was refluxed for 2-4 hrs to get corresponding alkylated esters (**2a-2c, 5a-5c, 13a-13b, 14a-14c, 15a-15e)** in quantitative yield (70-89%). These alkylated esters (**2a-2c, 5a-5c, 13a-13b, 14a-14c, 15a-15e)** (1eq.) were dissolved in methanol and hydroxylamine hydrochloride (10eq.) was added, followed by addition of potassium hydroxide (20eq.) at 0°C to -5°C and reaction mixture was stirred for 30-45 minutes at room temperature. After completion of reaction (TLC), 10% citric acid solution was added till pH is 7.0. The solution was extracted with ethylacetate/water and organic layer was dried over sodium sulphate and evaporated under reduced pressure to get crude product. The crude product was further purified by column chromatography over silica-gel (60-120 mesh) using 25-55% EtOAc/Hexane as eluent to get the corresponding alkylated hydroxamic acids **3a-3c, 6a-6c, 16a-16b, 17a-17c, 18a-18e** in promising yield (70-93%).

Typical Experimental Procedure for the synthesis of compounds (19-22):

A mixture of methyl salicylate **(4)** (1.1 gm, 7.0 mmol) and epichlorohydrin (3.0 ml, 35.0 mmol) was stirred magnetically at 30 °C. After 5 minute aqueous KOH (8ml) was slowly added and stirring continued for further 8 hrs. After the completion of the reaction, the reaction mixture was partitioned between ethyl acetate and water. The ethyl acetate layer was separated, dried over anhyd. Na_2SO_4 and evaporated under reduced pressure to give a crude mass. The crude mass was purified by column chromatography using SiO_2 (60-120 mesh) and hexane: ethyl acetate (4:1) as eluant to get the corresponding intermediate methyl 2-(oxiran-2-ylmethoxy)benzoate (**19**, 1.0 gm, 71%).

A mixture of the intermediate methyl 2-(oxiran-2-ylmethoxy)benzoate **(19)** (1.0gm, 4.01mmol) and sodium azide (0.31gm, 4.02mmol) in DMF (10 mL) was magnetically stirred at 60-80 °C for 6 hrs. Reaction was worked up by procedure given above and compound was purified by column

chromatography using SiO_2(60-120 mesh) and hexane: ethylacetate (7:3) as eluant to get methyl 2-(3-azido-2-hydroxypropoxy)benzoate (**20**, 0.8gm, 67%).

To a stirring solution of the above methyl 2-(3-azido-2-hydroxypropoxy) benzoate **(20)** (0.8gm, 3.0mml) and phenyl acetylene (0.32ml, 3.02mmol) in *t*-BuOH-water-acetone (2:2:1) (6mL), $CuSO_4$ (10mg 0.3mmol) and sodium ascorbate (26mg, 0.6mmol) was mixed properly and then slowly added. The stirring continued for further 5 h at 90 °C. After completion of reaction, reaction mixture was evaporated under reduced pressure and triturated with 10% MeOH/$CHCl_3$ and filtered through celite to give the methyl 2-(2-hydroxy-3-(4-phenyl-1H-1,2,3-triazol-1-yl)propoxy)benzoate (**21**, 0.7gm, 69%).

To a magnetically stirred solution of 2-(2-hydroxy-3-(4-phenyl-1*H*-1,2,3-triazol-1-yl)propoxy) benzoate **(21)** (0.7gm, 1.02mmol) in dry methanol at 0 to -5 °C hydroxylamine hydrochloride (1.3g, 19.02 mmol) was added, followed by addition of potassium hydroxide (2.2g, 39.0 mmol) and reaction mixture was stirred for 30-45 minute. After completion of reaction, 10% citric acid solution was added till pH 7. Reaction was worked up by procedure given above and crude mass was purified by column chromatography performed over Silica-gel (60-120 mesh) Chloroform:Methanol as eluant (2%MeOH in $CHCl_3$) to get the hydroxamate (**22,** 0.49g, 70%).

Spectral Data and Procedure of Hydroxamates

Methyl 1-(hexyloxy)-2-naphthoate (2a): It was obtained, by reaction of methyl 1-hydroxy-2-napthoate (1.4g, 6.93mmol) with 1-bromohexane (0.97ml, 6.93mmol) in the presence of potassium carbonte (0.95g, 6.93mmol) and tetrabutyl ammonium bromide (0.44g, 1.3mmol) in THF, as colorless syrup (1.60g, 81%); R_f = 0.49 (Ethyl acetate/hexane; 1:9); IR(neat), ν_{max} in cm^{-1}, 3222, 3021, 2350, 1715, 1216; ^1H NMR (200 MHz, $CDCl_3$) δ 8.03-7.78 (m, 2H, Ar-H), 7.81-7.76 (m, 1H, Ar-H), 7.54-7.51 (m, 3H, Ar-H), 4.08-4.00, (t, J = 6.7Hz, 2H, OCH_2), 3.79 (s,

3H, OCH_3), 1.99-1.89 (m, 2H, CH_2), 1.51-1.21 (m, 6H, CH_2), 0.93-0.89 (m, 3H, CH_3); ^{13}C NMR (50 MHz,$CDCl_3$) δ 167.9, 153.8, 137.0, 129.1, 128.4, 128.3, 127.2, 125.8, 125.3, 122.3, 74.3, 52.4, 29.6, 29.4, 29.3, 26.0, 22.8, 14.4; ESMS m/z = 287 $(M+H)^+$, Calculated elemental analysis for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74; Elemental analysis: C, 75.47; H, 7.71.

Methyl 1-(3,4-dichlorobenzyloxy)-2-naphthoate (2b): It was obtained, by reaction of methyl 1-hydroxy-2-napthoate (0.7g, 3.46mmol) with 3,4-dichloro benzyl bromide (0.82g, 3.46mmol) in the presence of potassium carbonte (0.47g, 3.46mmol) and tetrabutyl ammonium bromide (0.22g, 0.61mmol) in THF, as colorless syrup (0.98g, 79%); R_f = 0.54 (Ethyl acetate/hexane; 1:9); IR (neat), ν_{max} in cm^{-1}, 3115, 3041, 1723, 1355; ^1H NMR (200 MHz, $CDCl_3$) δ 8.09-7.37 (m, 9H, Ar-H), 4.98 (s, 2H, OCH_2), 3.94 (OCH_3); ^{13}C NMR (50MHz, $CDCl_3$) δ 171.4, 157.2, 140.4, 140.4, 135.2, 134.4, 134.4, 134.2, 132.3, 128.8, 127.3, 125.8, 78.1, 52.7; ESMS,m/z, 361 $(M+H)^+$; Calculated elemental analysis for $C_{19}H_{14}Cl_2O_3$: C, 63.18; H, 3.91, Elemental analysis found: C, 63.10; H, 3.87.

Methyl 1-(3-chlorobenzyloxy)-2-naphthoate (2c): It was obtained, by reaction of methyl 1-hydroxy-2-napthoate (0.9g, 4.41mmol) with 3-chloro benzyl bromide (0.61ml, 4.42mmol) in the presence of potassium carbonte (0.61g, 4.45mmol) and tetrabutyl ammonium bromide (0.28g, 0.80mmol) in THF, as colorless syrup (1.29g, 89%); R_f = 0.45 (Ethyl acetate/hexane; 1:9), IR(neat) ν_{max} in cm^{-1}, 3034, 2850, 1720, 1610, 1215; ^1H NMR (200 MHz, $CDCl_3$) δ 8.0-7.99 (d, J = 5.4 Hz, 1H, Ar-H), 7.82-7.78 (m, 2H, Ar-H), 7.54-7.47, (m, 4H, Ar-H), 7.40-7.17, (m, 3H, Ar-H), 4.88 (s, 2H, OCH_2), 3.88 (s, 3H, OCH_3); ^{13}C NMR (50MHz,$CDCl_3$) δ 169.5, 152.7, 130.6, 130.6, 128.9, 128.7, 128.3, 127.0, 126.3, 126.1, 125.2, 123.0, 119.4, 74.3, 52.0; ESMS, m/z, 326 $(M+H)^+$; Calculated elemental analysis for $C_{19}H_{15}ClO_3$: C, 69.84; H, 4.63; Elemental analysis found: C, 69.78; H, 4.54.

1-(Hexyloxy)-*N*-hydroxy-2-naphthamide (3a): It was obtained by reaction of correspoding methyl ester (1.1g, 3.8mmol) with the hydroxyl amine hydrochloride salt (2.66g, 38.3mmol) and poatassium hydroxide (4.29g, 76.6mmol) in methanol as white solid (0.93g, 85%); R_f = 0.56 (Ethyl acetate/hexane; 3:7); mp =104-106 ^{o}C ; IR (KBr), ν_{max} in cm^{-1}, 3422, 3021, 2361, 1648, 1216; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.10-7.79 (m, 2H, Ar-H), 7.82-7.78 (m, 1H, Ar-H), 7.58-7.50 (m, 3H, Ar-H), 4.08-4.01, (t, *J* = 6.6Hz, 2H, OCH_2), 2.03-1.89 (m, 2H, CH_2), 1.55-1.25 (m, 6H, CH_2), 0.95-0.89 (m, 3H, CH_3); ^{13}C NMR (50 MHz,$CDCl_3$) δ 164.9, 154.8, 137.0, 129.0, 128.5, 128.4, 127.0, 126.3, 125.0, 123.3, 77.2, 32.0, 30.6, 30.1, 26.0, 23.0, 14.4; ESMS m/z = 288 $(M+H)^{+}$; Calculated elemental analysis for $C_{17}H_{21}NO_3$: C, 71.06; H, 7.37; N, 4.87; Elemental analysis found: C, 71.0; H, 7.31; N, 4.82.

1-(3,4-Dichlorobenzyloxy)-*N*-hydroxy-2-naphthamide (3b): It was obtained by reaction of correspoding methyl ester (0.5g, 1.38mmol) with the hydroxyl amine hydrochloride salt (0.96g, 13.8mmol) and poatassium hydroxide (1.5g, 27.7mmol) in methanol as white solid (0.46g, 93%); R_f = 0.47 (Ethyl acetate/hexane; 3:7); mp =149-152 ^{o}C; IR(KBr),ν_{max} in cm^{-1}, 3215, 3061, 1633, 1355; ^{1}H NMR (200 MHz, $CDCl_3$ +CD_3OD) δ 8.12-7.39 (m, 9H, Ar-H), 5.03 (s, 2H, OCH_2); ^{13}C NMR (50MHz, $CDCl_3$+ CD_3OD) δ 169.4, 157.3, 140.7, 140.6, 135.0, 134.7, 134.4, 134.3, 132.4, 129.8, 127.5, 126.8, 80.1; ESMS,m/z, 362 $(M+H)^{+}$; Calculated elemental analysis for $C_{18}H_{13}Cl_2NO_3$: C, 59.69; H, 3.62; N, 3.87, Elemental analysis found: C, 59.64; H, 3.59; N, 3.81.

1-(3-Chlorobenzyloxy)-*N*-hydroxy-2-naphthamide (3c): It was obtained by reaction of correspoding methyl ester (0.8g, 2.41mmol) with the hydroxyl amine hydrochloride salt (1.70g, 24.5mmol) and poatassium hydroxide (2.74g, 49.0mmol) in methanol as white solid (0.69g, 87%); R_f = 0.51 (Ethyl acetate/hexane; 3:7); mp =99-101 ^{o}C; IR(KBr) ν_{max} in cm^{-1}, 3380, 3020, 2360, 1655, 1215; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.10-8.06 (d, *J* = 5.2 Hz, 1H, Ar-H), 7.88-7.78

(m, 2H, Ar-H), 7.64-7.47, (m, 4H, Ar-H), 7.41-7.17, (m, 3H, Ar-H), 4.98 (s, 2H, OCH_2); ^{13}C NMR (50MHz, $CDCl_3$) δ 164.5, 153.7, 130.6, 130.6, 129.3, 128.7, 128.6, 127.3, 126.8, 126.2, 125.5, 123.0, 119.7, 76.2, ESMS, m/z, 328 $(M+H)^+$; Calculated elemental analysis for $C_{18}H_{14}ClNO_3$: C, 65.96; H, 4.31; N, 4.27, Elemental analysis found: C, 65.89; H, 4.21; N, 4.19.

Methyl 2-(allyloxy)benzoate (5a): It was obtained, by reaction of methyl salicylate (1.0g, 6.5mmol) with allyl bromide (0.5ml, 6.51mmol) in the presence of potassium carbonte (0.92g, 6.57mmol) and tetrabutyl ammonium bromide (0.42g, 1.31mmol) in THF, as colorless syrup (0.96g, 80%); R_f = 0.52 (Ethyl acetate/hexane; 1:9), IR (neat), ν_{max} in cm^{-1}, 3204, 2912, 1724, 1267; ^1H NMR (200 MHz, CDCl3) δ 8.07-8.03 (m, 1H, Ar-H), 7.45-7.34 (m, 1H, Ar-H), 7.10-6.90 (m, 2H, Ar-H), 6.11-6.01 (m, 1H, -CH), 5.42-5.32 (m, 2H, $=CH_2$), 4.71-4.67 (d, J = 5.4Hz, 2H, OCH_2), 3.91 (s, 3H, OCH_3); ^{13}C NMR (50MHz,$CDCl_3$) δ 168.5, 156.3, 133.5, 133.0, 131.2, 131.0, 121.3, 119.2, 112.8, 72.1, 52.1; ESMS, m/z, 193 $(M+H)^+$; Calculated elemental analysis for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29, Elemental analysis found: C, 68.72; H, 6.24.

Methyl 2-(3,4-dichlorobenzyloxy)benzoate (5b): It was obtained, by reaction of methyl salicylate (1.4g, 9.2mmol) with 3,4-dichloro benzyl bromide (2.2g, 9.2mmol) in the presence of potassium carbonte (1.27g, 9.2mmol) and tetrabutyl ammonium bromide (0.59g, 1.8mmol) in THF, as colorless syrup (2.37g, 83%); R_f = 0.49 (Ethyl acetate/hexane; 1:9); IR (neat), ν_{max} in cm^{-1}; 3180, 3120, 2332, 1724, 1234; ^1H NMR (200 MHz, $CDCl_3$) δ 7.94-7.91 (m, 1H, Ar-H), 7.10-6.97 (m, 2H, Ar-H), 7.61-7.34(m, 4H, Ar-H), 5.19 (s, 2H, OCH_2), 3.92 (s, 3H, OCH_3); ^{13}C NMR (50MHz,$CDCl_3$) δ 169.9, 157.9, 139.4, 136.2, 133.3, 133.0, 132.3, 129.4, 124.6, 123.4, 116.3, 71.7, 53.0; ESMS, m/z, 310 $(M)^+$; Calculated elemental analysis for $C_{15}H_{12}Cl_2O_3$: C, 57.90; H, 3.89, Elemental analysis found: C, 57.88; H, 3.87.

Methyl 2-(3-chlorobenzyloxy)benzoate (5c): It was obtained, by reaction of methyl salicylate (1.2g, 7.8mmol) with 3-chloro benzyl bromide (1.01ml, 7.8mmol) in the presence of potassium carbonte (1.08g, 7.8mmol) and tetrabutyl ammonium bromide (0.50g, 1.5mmol) in THF, as colorless syrup (1.52g, 70%); R_f = 0.54 (Ethyl acetate/hexane; 1:9); IR (KBr), ν_{max} in cm^{-1}, 3380, 3021, 2361, 1717, 1216; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 7.7 Hz, 1H, Ar-H), 6.56-6.26 (m, 5H, Ar-H), 6.15-6.02, (m, 2H, Ar-H), 4.23 (s, 2H, OCH_2), 3.81 (s, 3H, OCH_3); ^{13}C NMR (50 MHz, $CDCl_3$), δ 171.6, 159.2, 148.5, 134.4, 132.1, 122.7, 121.8, 120.5, 115.6, 71.6, 52.0; ESMS, m/z, 242 $(M+H)^+$; Calculated elemental analysis for $C_{15}H_{13}ClO_3$: C, 65.11; H, 4.74, Elemental analysis found, C, 65.07; H, 4.69.

Allyloxy-*N*-hydroxy-benzamide (6a): It was obtained by reaction of correspoding methyl ester (0.90g, 4.68mmol) with the hydroxyl amine hydrochloride salt (3.25g, 46.8mmol) and poatassium hydroxide (5.25g, 93.7mmol) in methanol as white solid (0.63g, 70%); R_f = 0.51 (Ethyl acetate/hexane; 3:7), mp = 120-122 ^{o}C; IR(KBr), ν_{max} in cm^{-1}, 3300, 2904, 1625, 1600, 1267; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.09-8.04 (m, 1H, Ar-H), 7.47-7.38 (m, 1H, Ar-H), 7.10-6.95 (m, 2H, Ar-H), 6.16-6.02 (m, 1H, -CH), 5.49-5.34 (m, 2H, $=CH_2$), 4.71-4.69 (d, *J* = 5.4Hz, 2H, OCH_2) ; ^{13}C NMR (50MHz,$CDCl_3$) δ 164.5, 156.4, 134.5, 133.3, 132.2, 131.8, 121.8, 119.4, 112.9, 70.1; ESMS, m/z, 194 $(M+H)^+$; Calculated elemental analysis for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25, Elemental analysis found: C, 62.12; H, 5.73; N, 7.21.

2-(3,4-Dichloro-benzyloxy)-*N*-hydroxy-benzamide (6b): It was obtained by reaction of correspoding methyl ester (1.2g, 3.85mmol) with the hydroxyl amine hydrochloride salt (2.68g, 38.5mmol) and poatassium hydroxide (4.32g, 77.1mmol) in methanol as white solid (1.10g, 92%); R_f= 0.49 (Ethyl acetate/hexane; 3:7); mp = 138-140^{o}C; IR(KBr), ν_{max} in cm^{-1}, 3387, 3120, 2362, 1645, 1234; ^{1}H NMR (200 MHz, $CDCl_3$+CD_3OD) δ 7.95-7.92 (m, 1H, Ar-H), 7.12-6.99

(m, 2H, Ar-H), 7.65-7.31 (m, 4H, Ar-H), 5.19 (s, 2H, OCH_2); ^{13}C NMR (50MHz,$CDCl_3$+ CD_3OD) δ 167.7, 158.9, 139.5, 136.0, 134.2, 133.9, 132.4, 129.8, 124.8, 123.6, 116.1, 72.7; ESMS, m/z, 311 $(M+H)^+$; Calculated elemental analysis for $C_{14}H_{11}Cl_2NO_3$: C, 53.87; H, 3.55; N, 4.49, Elemental analysis found: C, 53.84; H, 3.52; N, 4.46.

2-(3-Chloro-benzyloxy)-*N*-hydroxy-benzamide (6c): It was obtained by reaction of correspoding methyl ester (1.0g, 3.6mmol) with the hydroxyl amine hydrochloride salt (2.5g, 36.2mmol) and poatassium hydroxide (4.05g, 72.4mmol) in methanol as white solid (0.87g, 87%); R_f = 0.52 (Ethyl acetate/hexane; 3:7), mp = 92-95 ^oC; IR (KBr), ν_{max} in cm^{-1}, 3448, 3021, 2361, 1645, 1216; ^1H NMR (200 MHz, $CDCl_3$) δ 7.15 (dd, *J* = 7.7 Hz, 1H, Ar-H), 6.56-6.26 (m, 5H, Ar-H), 6.15-6.02, (m, 2H, Ar-H), 4.23 (s, 2H, OCH_2); ^{13}C NMR (50 MHz, $CDCl_3$), δ 166.6, 159.2, 148.5, 134.4, 132.1, 122.7, 121.8, 120.5,115.6, 71.6; ESMS *m/z* = 278 $(M + H)^+$ Calculated elemental analysis for $C_{14}H_{12}ClNO_3$: C, 60.55; H, 4.36; N, 5.04, Elemental analysis found: C, 60.51; H, 4.32; N, 5.14.

Methyl 3-(allyloxy)-2-hydroxybenzoate (10a): It was obtained, by reaction of methyl 2,3-dihydroxybenzoate (1.0g, 5.9mmol) with allyl bromide (0.82ml, 9.5mmol) in presence of potassium carbonte (0.82g, 5.9mmol) and tetrabutyl ammonium bromide (0.38g, 1.2mmol) in acetone, as colorless syrup (0.96g, 78%); R_f = 0.45 (Ethyl acetate/hexane; 1:9); ^1H NMR (300 MHz, $CDCl_3$) δ 10.94 (s,1H, ArOH), 7.31 (t, *J* = 4.6 Hz, 1H, Ar-H), 7.02-7.00 (m, 2H, Ar-H), 6.15-6.05 (m, 1H, CH), 5.32-5.18 (m, 2H, CH_2), 4.58-4.55 (m, 2H, CH_2), 3.86 (s, 3H, OCH_3); ESMS, m/z, 209 $(M+H)^+$; Calculated elemental analysis for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; Elemental analysis found, C, 63.40; H, 5.78.

Methyl 3-(benzyloxy)-2-hydroxybenzoate (10b): It was obtained, by reaction of methyl 2,3-dihydroxybenzoate (1.0g, 5.9mmol) with benzyl bromide (0.84ml, 7.13mmol) in presence of

potassium carbonte (0.82g, 5.9mmol) and tetrabutyl ammonium bromide (0.38g, 1.2mmol) in acetone, as white solid (1.13g, 74%); R_f = 0.50 (Ethyl acetate/hexane; 1:9); m.p. = 86-88 °C.

Methyl 4-(allyloxy)-2-hydroxybenzoate (11a): It was obtained, by reaction of methyl 2,4-dihydroxybenzoate (1.0g, 5.9mmol) with allyl bromide (0.82ml, 9.5mmol) in presence of potassium carbonte (0.82g, 5.9mmol) and tetrabutyl ammonium bromide (0.38g, 1.2mmol) in acetone, as colorless syrup (0.94g, 76%); R_f = 0.45 (Ethyl acetate/hexane; 1:9).

Methyl 4-(2, 5-dichlorobenzyloxy)-2-hydroxybenzoate (11b): It was obtained, by reaction of methyl 2,4-dihydroxybenzoate (0.55g, 3.27mmol) with 2,5-dicholorobenzyl bromide (0.79g, 3.27mmol) in the presence of potassium carbonate (0.45g, 3.27mmol) and tetrabutyl ammonium bromide (0.21g, 0.26mmol) in acetone, as white solid (0.78g, 74%); R_f = 0.6 (Ethyl acetate/hexane; 1:9), ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.94 (s,1H, ArOH), 7.78-7.75 (t, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 7.35-7.25 (m, 2H, Ar-H), 6.54-6.52 (m, 2H, Ar-H), 5.14 (s, 2H, OCH_2), 3.94 (s, 3H, OCH_3); ESMS, m/z, 327 $(M+H)^+$; Calculated elemental analysis for $C_{15}H_{12}Cl_2O_4$: C, 55.07; H, 3.70 , Elemental analysis found: C, 55.02; H, 3.67.

Methyl 4-(3-bromobenzyloxy)-2-hydroxybenzoate (11c): It was obtained, by reaction of methyl 2,4-dihydroxybenzoate (0.55g, 3.27mmol) with 4-bromo benzyl bromide (0.82g, 3.27mmol) in the presence of potassium carbonate (0.45g, 3.27mmol) and tetrabutyl ammonium bromide (0.21g, 0.65mmol) in acetone, as white solid (0.85g, 78%); R_f = 0.6 (Ethyl acetate/hexane; 1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.94 (s,1H, ArOH), 7.75-7.72 (d, 1H,*J*= 9.54 Hz, Ar-H), 7.54-7.51 (d, 2H, *J*= 8.28 Hz, Ar-H), 7.31-7.28 (d, 2H, *J*= 8.37 Hz, Ar-H), 6.49-6.46 (m, 2H, Ar-H), 5.03 (s, 2H, OCH_2), 3.96 (s, 3H, OCH_3); ^{13}C NMR (50MHz,$CDCl_3$) δ 170.1, 164.2, 163.8, 135.0, 131.7, 131.2, 128.9, 122.2, 107.9, 105.8, 101.6, 69.2, 51.8; ESMS,

m/z, 337 $(M+H)^+$; Calculated elemental analysis for $C_{15}H_{13}BrO_4$: C, 53.43; H, 3.89, Elemental analysis found: C, 53.47; H, 3.84.

Methyl 5-(allyloxy)-2-hydroxybenzoate (12a): It was obtained, by reaction of methyl 2,5-dihydroxybenzoate (1.0g, 5.9mmol) with allyl bromide (0.82ml, 9.5mmol) in presence of potassium carbonte (0.82g, 5.9mmol) and tetrabutyl ammonium bromide (0.38g, 1.2mmol) in acetone, as colorless syrup (1.0g, 81%); R_f = 0.45 (Ethyl acetate/hexane; 1:9).

Methyl 2-hydroxy-5-(prop-2-ynyloxy)benzoate (12b): It was obtained, by reaction of methyl 2,5-dihydroxybenzoate (1.0g, 5.9mmol) with propargyl bromide (0.84ml, 9.5mmol) in presence of potassium carbonte (0.82g, 5.9mmol) and tetrabutyl ammonium bromide (0.38g, 1.2mmol) in acetone, as colorless syrup (0.97g, 79%); R_f = 0.55 (Ethyl acetate/hexane; 1:9).

Methyl 5-(benzyloxy)-2-hydroxybenzoate (12c): It was obtained, by reaction of methyl 2,5-dihydroxybenzoate (1.0g, 5.9mmol) with benzyl bromide (0.84ml, 7.13mmol) in presence of potassium carbonte (0.82g, 5.9mmol) and tetrabutyl ammonium bromide (0.38g, 1.2mmol) in acetone, as white solid (1.16g, 76%); R_f = 0.50 (Ethyl acetate/hexane; 1:9); m.p. = 91-93°C.

Methyl 5-(2, 5-dichlorobenzyloxy)-2-hydroxybenzoate (12d): It was obtained, by reaction of methyl 2,5-dihydroxybenzoate (0.8g, 4.76mmol) with 2,5-dicholorobenzyl bromide (1.4g, 4.76mmol) in the presence of potassium carbonate (0.78g, 4.76mmol) and tetrabutyl ammonium bromide (0.31g, 0.95mmol) in acetone, as white solid (0.68g, 76%); R_f = 0.6 (Ethyl acetate/hexane; 1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.36 (s,1H, ArOH), 7.58-7.57 (d, 1H, *J*= 2.1 Hz, Ar-H), 7.37-7.13 (m, 4H, Ar-H), 6.94-6.91 (d, 1H, *J*= 9.21 Hz, Ar-H), 5.04 (s, 2H, OCH_2), 3.96 (s, 3H, OCH_3); ^{13}C NMR (50MHz,$CDCl_3$) δ 170.1, 156.93, 150.5, 136.5, 133.2, 130.5, 130.4, 129.0, 128.6, 124.6, 118.9, 113.7, 112.0, 67.4, 52.3; ESMS, m/z, 327 $(M+H)^+$;

Calculated elemental analysis for $C_{15}H_{12}Cl_2O_4$: C, 55.07; H, 3.70 , Elemental analysis found: C, 55.09; H, 3.67.

Methyl 5-(4-bromobenzyloxy)-2-hydroxybenzoate (12e): It was obtained, by reaction of methyl 2,5-dihydroxybenzoate (0.55g, 3.27mmol) with 4-bromo benzyl bromide (0.82g, 3.27mmol) in the presence of potassium carbonte (0.45g, 3.27mmol) and tetrabutyl ammonium bromide (0.21g, 0.65mmol) in acetone, as white solid (0.83g, 76%); R_f = 0.6 (Ethyl acetate/hexane; 1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.30 (s,1H, ArOH), 7.49-7.46 (d, 1H, *J*= 8.28 Hz, Ar-H), 7.31-7.25 (m, 3H, Ar-H), 7.10-7.06 (m, 1H, Ar-H), 6.90-6.87 (d, 1H, *J*= 9.03 Hz, Ar-H) 4.93 (s, 2H, OCH_2), 3.93 (s, 3H, OCH_3); ESMS, m/z, 337 $(M+H)^+$; Calculated elemental analysis for $C_{15}H_{13}BrO_4$: C, 53.43; H, 3.89, Elemental analysis found: C, 53.39; H, 3.93.

Methyl 2,3-bis(allyloxy)benzoate (13a): It was obtained, by reaction of methyl 3-(allyloxy)-2-hydroxybenzoate (0.7g, 3.4mmol) with allyl bromide (0.46ml, 5.38mmol) in presence of potassium carbonte (0.46g, 3.4mmol) and tetrabutyl ammonium bromide (0.21g, 0.67mmol) in reflluxing THF, as colorless syrup (0.68g, 82%); R_f= 0.45 (Ethyl acetate/hexane; 1:9).

Methyl 2-(allyloxy)-3-(benzyloxy)benzoate (13b): It was obtained, by reaction of methyl 3-(benzyloxy)-2-hydroxybenzoate (0.7g, 2.7mmol) with allyl bromide (0.37ml, 4.33mmol) in presence of potassium carbonte (0.37g, 2.7mmol) and tetrabutyl ammonium bromide (0.17g, 0.54mmol) in reflluxing THF, as colorless syrup (0.69g, 86%); R_f = 0.40 (Ethyl acetate/hexane; 1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.78 (s, 1H, Ar-H), 7.49-7.38 (m, 5H, Ar-H), 7.01-6.87 (m, 2H, Ar-H), 6.10-6.03 (m, 1H, CH), 5.49-5.33 (m, 2H, CH_2), 4.87 (s, 2H, OCH_2), 4.56 (s, 2H, OCH_2) 3.87 (s, 3H, OCH_3); ESMS, m/z, 209 $(M+H)^+$; Calculated elemental analysis for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; Elemental analysis found, C, 63.40; H, 5.78.

Methyl 2,4-bis(allyloxy)benzoate (14a): It was obtained, by reaction of methyl 4-(allyloxy)-2-hydroxybenzoate (0.7g, 3.4mmol) with allyl bromide (0.46ml, 5.38mmol) in presence of potassium carbonte (0.46g, 3.4mmol) and tetrabutyl ammonium bromide (0.21g, 0.67mmol) in reflluxing THF, as colorless syrup (0.65g, 78%); R_f = 0.45 (Ethyl acetate/hexane; 1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (s, 1H, Ar-H), 7.02-7.00 (m, 2H, Ar-H), 6.15-5.97 (m, 2H, 2xCH), 5.44-5.18 (m, 4H, 2xCH_2), 4.59-4.56 (m, 4H, 2xOCH_2), 3.89 (s, 3H, OCH_3); ESMS, m/z, 209 $(M+H)^+$; Calculated elemental analysis for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; Elemental analysis found, C, 63.40; H, 5.78.

Methyl 2-(allyloxy)-4-(2, 5-dichlorobenzyloxy) benzoate (14b): It was obtained, by refluxing of methyl 4-(2,5-dichlorobenzyloxy)-2-hydroxybenzoate (0.75g, 2.23mmol) with allyl bromide (0.19ml, 2.23mmol) in the presence of potassium carbonate (0.37g, 2.23mmol) and tetrabutyl ammonium bromide (0.14g, 0.44mmol) in THF, as white solid (0.73g, 86%); R_f = 0.5 (Ethyl acetate/hexane; 1:9), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88-7.85 (t, 1H, Ar-H) 7.56-7.55 (d, 1H, *J*=1.92 Hz, Ar-H) 7.36-7.15 (m, 2H, Ar-H), 6.58-6.56 (t, 2H, Ar-H), 6.14-6.01 (m, 1H, -CH), 5.58-5.52 (dd, 1H, *J*= 1.41 Hz, =CH_2) 5.34-5.30 (dd, 1H, *J*= 1.32 Hz, =CH_2), 5.12 (s, 2H, OCH_2), 4.63-4.62 (m, 2H, OCH_2), 3.88 (s, 3H, OCH_3); ^{13}C NMR (50MHz,$CDCl_3$) δ 165.6, 162.3, 160.2, 135.8, 133.9, 133.2, 132.4, 130.4, 129.0, 128.4, 117.4, 113.7, 105.5, 101.0, 69.3, 66.4, 51.5, ESMS, m/z, 367 $(M+H)^+$; Calculated elemental analysis for $C_{18}H_{16}Cl_2O_4$: C, 58.87; H, 4.39, Elemental analysis found: C, 58.89; H, 4.34.

Methyl 2-(allyloxy)-4-(3-bromobenzyloxy) benzoate (14c): It was obtained, by refluxing of methyl 4-(3-bromobenzyloxy)-2-hydroxybenzoate (0.6g, 1.72mmol) with allyl bromide (0.15ml, 1.72mmol) in the presence of potassium carbonte (0.30g, 1.72mmol) and tetrabutyl ammonium bromide (0.11g, 0.34mmol) in THF, as white solid (0.54g, 81%); R_f = 0.5 (Ethyl acetate/hexane;

1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86-7.83 (d, 1H, *J*= 8.61 Hz, Ar-H) 7.54-7.51 (d, 2H, *J*= 8.31Hz, Ar-H) 7.30-7.28 (d, 2H, *J*= 8.34 Hz, Ar-H), 6.55-6.51 (t, 2H, Ar-H), 6.12-5.99 (m, 1H, CH), 5.56-5.06 (dd, 1H, *J*= 1.50 Hz, $=CH_2$) 5.33-5.29 (dd, 1H, *J*= 1.50 Hz, $=CH_2$), 5.04 (s, 2H, OCH_2), 4.60-4.58 (m, 2H, OCH_2), 3.87 (s, 3H, OCH_3); ESMS, m/z, 377 $(M+H)^+$; Calculated elemental analysis for $C_{18}H_{17}BrO_4$: C, 57.31; H, 4.54, Elemental analysis found: C, 57.34; H, 4.51.

Methyl 2,5-bis(allyloxy)benzoate (15a): It was obtained, by reaction of methyl 5-(allyloxy)-2-hydroxybenzoate (0.7g, 3.4mmol) with allyl bromide (0.46ml, 5.38mmol) in presence of potassium carbonte (0.46g, 3.4mmol) and tetrabutyl ammonium bromide (0.21g, 0.67mmol) in reflluxing THF, as colorless syrup (0.63g, 76%); R_f= 0.45 (Ethyl acetate/hexane; 1:9).

Methyl 2-(allyloxy)-5-(prop-2-ynyloxy)benzoate (15b): It was obtained, by reaction of methyl 2-hydroxy-3-(prop-2-ynyloxy)benzoate (0.7g, 3.4mmol) with allyl bromide (0.47ml, 5.40mmol) in presence of potassium carbonte (0.46g, 3.4mmol) and tetrabutyl ammonium bromide (0.21g, 0.67mmol) in reflluxing THF, as colorless syrup (0.67g, 81%); R_f = 0.40 (Ethyl acetate/hexane; 1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.69 (s, 1H, Ar-H), 7.47 (d, *J*= 4.34 Hz, 1H, Ar-H), 7.37 (d, *J*= 4.32 Hz, 1H, Ar-H), 5.97-5.93 (m, 1H, CH), 5.32-5.26 (m, 2H, CH_2), 5.02 (s, 2H, OCH_2), 4.65 (s, 2H, OCH_2), 3.93 (s, 3H, OCH_3), 3.41 (s, 1H, CH), ; ESMS, m/z, 209 $(M+H)^+$; Calculated elemental analysis for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; Elemental analysis found, C, 63.40; H, 5.78.

Methyl 2-(allyloxy)-5-(benzyloxy)benzoate (15c): It was obtained, by reaction of methyl 5-(benzyloxy)-2-hydroxybenzoate (0.7g, 2.7mmol) with allyl bromide (0.37ml, 4.33mmol) in presence of potassium carbonte (0.37g, 2.7mmol) and tetrabutyl ammonium bromide (0.17g, 0.54mmol) in reflluxing THF, as colorless syrup (0.67g, 83%); R_f = 0.40 (Ethyl acetate/hexane;

1:9); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 1H, Ar-H), 7.39-7.27 (m, 5H, Ar-H), 7.00-6.84 (m, 2H, Ar-H), 6.06-6.00 (m, 1H, CH), 5.47-5.31 (m, 2H, CH_2), 5.00 (s, 2H, OCH_2), 4.61 (s, 2H, OCH_2) 3.96 (s, 3H, OCH_3); ESMS, m/z, 209 $(M+H)^+$; Calculated elemental analysis for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81; Elemental analysis found, C, 63.40; H, 5.78.

Methyl 2-(allyloxy)-5-(2, 5-dichlorobenzyloxy) benzoate (15d): It was obtained, by reaction of methyl 5-(2,5-dichlorobenzyloxy)-2-hydroxybenzoate (0.36g, 1.07mmol) with allyl bromide (0.1ml, 1.07mmol) in the presence of potassium carbonate (0.18g, 1.07mmol) and tetrabutyl ammonium bromide (0.07g, 0.21mmol) in THF, as white solid (0.25g, 83%); R_f = 0.5 (Ethyl acetate/hexane; 1:9), IR (neat); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46-7.45 (d, 1H, *J*=3.06, Ar-H) 7.35-7.32 (d, 1H, *J*=7.35 Hz, Ar-H) 7.27-7.24 (m, 3H, Ar-H), 7.10-7.03 (m, 1H, Ar-H) 6.95-6.92 (d, 1H, *J*= 9.18 Ar-H), 6.13-6.00 (m, 1H, -CH), 5.53-5.47 (d, 1H, *J*= 17.16 Hz, $=CH_2$) 5.32-5.29 (d, 1H, *J*= 9.81 Hz, $=CH_2$), 5.09 (s, 2H, OCH_2), 4.60-4.58 (d, 2H, *J*= 4.29 Hz, OCH_2), 3.93 (s, 3H, OCH_3); ^{13}C NMR (50MHz,$CDCl_3$) δ 166.0, 153.0, 136.4, 133.1,133.0, 130.3, 130.0, 128.8, 128.5, 121.5, 120.0, 117.3, 115.8, 70.4, 67.1, 51.9; ESMS, m/z, 367 $(M+H)^+$; Calculated elemental analysis for $C_{18}H_{16}Cl_2O_4$: C, 58.87; H, 4.39, Elemental analysis found: C, 58.83; H, 4.42.

Methyl 2-(allyloxy)-5-(4-bromobenzyloxy) benzoate (15e): It was obtained, by reaction of methyl 5-(4-bromobenzyloxy)-2-hydroxybenzoate (0.65g, 1.87mmol) with allyl bromide (0.16ml, 1.87mmol) in the presence of potassium carbonte (0.26g, 1.87mmol) and tetrabutyl ammonium bromide (0.12g, 0.37mmol) in THF, as white solid (0.56g, 78%); R_f = 0.5 (Ethyl acetate/hexane; 1:9); ^{1}H NMR (300 MHz, CDCl3) δ 7.53-7.50 (d, 2H, *J*= 8.41 Hz, ArH) 7.42-7.41 (d, 1H, *J*= 3.50 Hz, ArH) 7.32-7.26 (m, 2H, ArH), 7.05-7.01 (m, 1H, ArH), 6.12-6.00 (m, 1H, CH), 5.51-5.45 (d, 1H, *J*= 16.11 Hz, $=CH_2$) 5.31-5.28 (d, 1H, *J*= 11.02 Hz, $=CH_2$), 5.00 (s,

2H, OCH_2), 4.58-4.57 (d, 2H, *J*= 4.24 Hz, OCH_2), 3.91 (s, 3H, OCH_3); ESMS, m/z, 377 $(M+H)^+$; Calculated elemental analysis for $C_{18}H_{17}BrO_4$: C, 57.31; H, 4.54, Elemental analysis found: C, 57.28; H, 4.59.

2,3-bis(allyloxy)-*N*-hydroxybenzamide (16a): It was obtained by reaction of methyl 2,3-bis(allyloxy)benzoate (0.5g, 2.0mmol) with the hydroxyl amine hydrochloride salt (1.39g, 20.15mmol) and poatassium hydroxide (2.25g, 40.3mmol) in methanol as white solid (0.40g, 80%); R_f = 0.50 (Ethyl acetate/hexane; 4:6), mp = 100-102 ^oC; IR (KBr), ν_{max} in cm^{-1}, 3437, 1651, 1575, 1217, 1024, 769; ^1H NMR (300 MHz, $CDCl_3$) δ 10.42 (bs, 1H, NH), 7.70 (d, *J* = 6.9 Hz, 1H, Ar-H), 7.28-7.02 (m, 2H, Ar-H), 6.18-6.00 (m, 2H, 2xCH), 5.46-5.30 (m, 5H, $2xCH_2$, NH), 4.67 (d, *J* = 5.91 Hz, 2H, OCH_2), 4.67 (d, *J* = 4.77 Hz, 2H, OCH_2); ^{13}C NMR (50 MHz, $CDCl_3$+CCl_4), δ 163.3, 151.3, 146.0, 132.6, 124.4, 122.8, 120.0, 117.9, 117.4, 74.7, 69.8; ESMS *m/z* = 250 $(M + H)^+$ Calculated elemental analysis for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62, Elemental analysis found: C, 62.59; H, 6.10; N, 5.57.

2-(allyloxy)-3-(benzyloxy)-*N*-hydroxybenzamide (16b): It was obtained by reaction of methyl 2-(allyloxy)-3-(benzyloxy)benzoate (0.5g, 1.6mmol) with the hydroxyl amine hydrochloride salt (1.16g, 16.7mmol) and poatassium hydroxide (1.87g, 33.5mmol) in methanol as white solid (0.43g, 86%); R_f= 0.50 (Ethyl acetate/hexane; 5:5), mp = 96-98 ^oC; IR (KBr), ν_{max} in cm^{-1}, 3386, 1645, 1217, 1023, 768, ^1H NMR (300 MHz, $CDCl_3$) δ 10.21 (s, 1H, NH), 7.77 (s, 1H, Ar-H), 7.51-7.40 (m, 5H, Ar-H), 6.97-6.85 (m, 2H, Ar-H), 6.07-5.99 (m, 1H, CH), 5.53-5.37 (m, 2H, CH_2), 4.83 (s, 2H, OCH_2), 4.59 (s, 2H, OCH_2); ESMS *m/z* = 300 $(M + H)^+$ Calculated elemental analysis for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72; N, 4.68, Elemental analysis found: C, 68.15; H, 5.70; N, 4.62.

2,4-bis(allyloxy)-*N*-hydroxybenzamide (17a): It was obtained by reaction of methyl 2,4-bis(allyloxy)benzoate (0.5g, 2.0mmol) with the hydroxyl amine hydrochloride salt (1.39g, 20.15mmol) and poatassium hydroxide (2.25g, 40.3mmol) in methanol as white solid (0.39g, 78%); R_f = 0.45 (Ethyl acetate/hexane; 4:6), mp = 104-106 ^{o}C; IR (KBr), ν $_{max}$ in cm^{-1}, 3431, 1647, 1573, 1210, 1021, 768; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.23 (s,1H, NH), 7.36 (s, 1H, Ar-H), 7.06-7.01 (m, 2H, Ar-H), 6.18-5.99 (m, 2H, 2xCH), 5.32-5.06 (m, 4H, 2xCH_2), 4.61-4.59 (m, 4H, 2xOCH_2); ^{13}C NMR (50 MHz, $CDCl_3$+CCl_4), δ 163.7, 150.9, 150.1, 132.7, 132.0, 120.8, 119.1, 116.6, 116.0, 113.8, 70.2, 69.3; ESMS *m/z* = 250 $(M + H)^+$ Calculated elemental analysis for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62, Elemental analysis found: C, 62.58; H, 6.03; N, 5.57.

2-(allyloxy)-4-(2, 5-dichlorobenzyloxy)-*N*-hydroxybenzamide (17b): It was obtained, by reaction of methyl 2-(allyloxy)-4-(2,5-dichlorobenzyloxy)benzoate (0.7g, 1.91mmol) with hydroxylamine hydrochloride (1.30g, 19.1mmol) in the presence of potassium hydroxide (2.20g, 38.2mmol) at 0˚C in methanol, as brown solid (0.53g, 76%); R_f = 0.3 (Ethyl acetate/hexane; 4:6), mp = 136-138 ^{o}C; IR (KBr), ν $_{max}$ in cm^{-1}, 3401, 1638, 1487, 1211, 1010, 771; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.12 (s,1H, NH), 8.18-8.15 (d, 1H, *J*=8.79 Hz, Ar-H), 7.55 (s, 1H, Ar-H), 7.37-7.34 (m, 2H, Ar-H), 6.74-6.58 (m, 2H, Ar-H), 6.15-6.08 (m, 1H, CH), 5.52-5.42 (m, 2H, =CH_2) 5.16 (s, 2H, OCH_2), 4.79-4.77 (d, 1H, *J*= 5.31, OH), 4.72-4.70 (d, 2H, *J*= 5.07, OCH_2); ^{13}C NMR (50 MHz, $CDCl_3$+CCl_4) δ 161.9, 157.5, 135.6, 133.6, 133.2, 131.5, 130.4, 129.2, 128.5, 120.0, 107.3, 106.6, 100.3, 70.0, 66.6; ESMS, m/z, 368.2 $(M+H)^+$; Calculated elemental analysis for $C_{17}H_{15}Cl_2NO_4$: C, 55.45; H, 4.11; N, 3.80, Elemental analysis found: C, 55.41; H, 4.14; N, 3.83.

2-(allyloxy)-4-(3-bromobenzyloxy)-*N*-hydroxybenzamide (17c): It was obtained, by reaction of methyl 2-(allyloxy)-4-(3-bromobenzyloxy)benzoate (0.36g, 0.98mmol) with hydroxylamine

hydrochloride (0.68g, 9.8mmol) in the presence of potassium hydroxide (1.10g,19. 6mmol) at 0˚C in methanol, as brown solid (0.28g, 75%); R_f = 0.3 (Ethyl acetate/hexane; 4:6), mp = 118-120 ^{o}C; IR (KBr), ν $_{max}$ in cm^{-1}, 3406, 1645, 1493, 1219, 1009, 769; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.14 (s, 1H, NH), 8.14-8.11 (d, 1H, *J*=8.40 Hz, Ar-H), 7.53-7.51 (d, 2H, *J*= 7.38 Hz, Ar-H), 7.31-7.28 (d, 2H, *J*= 8.52 Hz, Ar-H), 6.67-6.49 (m, 2H, Ar-H), 6.16-6.01 (m, 1H, CH), 5.51-5.37 (m, 2H, =CH_2) 5.04 (s, 2H, OCH_2), 4.73 (s, 1H, OH), 4.66-4.64 (d, 2H, *J*= 6.15 Hz, OCH_2); ESMS, m/z, 378.2 $(M+H)^+$; Calculated elemental analysis for $C_{17}H_{16}BrNO_4$: C, 53.99; H, 4.26; N, 3.70, Elemental analysis found: C, 53.94; H, 4.21; N, 3.72.

2,5-bis(allyloxy)-*N*-hydroxybenzamide (18a): It was obtained by reaction of methyl 2,5-bis(allyloxy)benzoate (0.5g, 2.0mmol) with the hydroxyl amine hydrochloride salt (1.39g, 20.15mmol) and poatassium hydroxide (2.25g, 40.3mmol) in methanol as white solid (0.39g, 78%); R_f = 0.55 (Ethyl acetate/hexane; 5:5), mp = 101-103 ^{o}C; IR (KBr), ν $_{max}$ in cm^{-1}, 3376, 1647, 1491, 1210, 1006, 766; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 10.39 (bs, 1H, NH), 7.68 (s, 1H, Ar-H), 6.95-6.87 (m, 2H, Ar-H), 6.04-6.03 (m, 2H, 2xCH), 5.45-5.23 (m, 5H, 2xCH_2, NH), 4.64 (s, 2H, OCH_2), 4.51 (s, 2H, OCH_2); ^{13}C NMR (50 MHz, $CDCl_3$+CCl_4), δ 163.3, 151.1, 150.4, 133.0, 132.0, 120.3, 119.4, 117.6, 116.1, 114.1, 70.6, 69.2; ESMS *m/z* = 250 $(M + H)^+$ Calculated elemental analysis for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62, Elemental analysis found: C, 62.55; H, 6.01; N, 5.58.

2-(allyloxy)-*N*-hydroxy-5-(prop-2-ynyloxy)benzamide (18b): It was obtained by reaction of methyl 2-(allyloxy)-3-(prop-2-ynyloxy)benzoate (0.5g, 2.0mmol) with the hydroxyl amine hydrochloride salt (1.40g, 20.31mmol) and poatassium hydroxide (2.27g, 40.6mmol) in methanol as white solid (0.37g, 74%); R_f = 0.50 (Ethyl acetate/hexane; 5:5), mp = 98-100 ^{o}C; IR (KBr), ν $_{max}$ in cm^{-1}, 3541, 1623, 1217, 1024, 771; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ ^{1}H NMR

(300 MHz, $CDCl_3$) δ 10.23 (s, 1H, NH), 7.70 (s, 1H, Ar-H), 7.51 (d, *J*= 4.32 Hz, 1H, Ar-H), 7.43 (d, *J*= 4.36 Hz, 1H, Ar-H), 5.95-5.91 (m, 1H, CH), 5.28-5.21 (m, 2H, CH_2), 4.97 (s, 2H, OCH_2), 4.67 (s, 2H, OCH_2), 3.37 (s, 1H, CH), ESMS *m/z* = 248 $(M + H)^+$ Calculated elemental analysis for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67, Elemental analysis found: C, 63.09; H, 5.25; N, 5.63.

2-(allyloxy)-5-(benzyloxy)-*N*-hydroxybenzamide (18c): It was obtained by reaction of methyl 2-(allyloxy)-3-(benzyloxy)benzoate (0.5g, 1.6mmol) with the hydroxyl amine hydrochloride salt (1.16g, 16.7mmol) and poatassium hydroxide (1.87g, 33.5mmol) in methanol as white solid (0.40g, 81%); R_f= 0.55 (Ethyl acetate/hexane; 5:5), mp = 95-97 ^oC; IR (KBr), ν $_{max}$ in cm^{-1}, 3445, 1646, 1491, 1215, 1011, 769; ^1H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 10.36 (bs, 1H, NH), 7.78 (s, 1H, Ar-H), 7.36-7.23 (m, 6H, Ar-H), 7.00-6.84 (m, 2H, Ar-H), 6.07-6.01 (m, 1H, CH), 5.44-5.34 (m, 2H, CH_2), 5.03 (s, 2H, OCH_2), 4.63 (s, 2H, OCH_2); ^{13}C NMR (50 MHz, $CDCl_3$+CCl_4), δ 161.3, 153.1, 150.4, 136.6, 132.0, 128.5, 127.9, 127.5, 120.5, 119.6, 114.2, 70.5; ESMS *m/z* = 300 $(M + H)^+$ Calculated elemental analysis for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72; N, 4.68, Elemental analysis found: C, 68.17; H, 5.68; N, 4.63.

2-(allyloxy)-5-(2, 5-dichlorobenzyloxy)-*N*-hydroxybenzamide (18d): It was obtained, by reaction of methyl 2-(allyloxy)-5-(2,5-dichlorobenzyloxy)benzoate (0.30g, 0.81mmol) with hydroxylamine hydrochloride (0.6g, 8.10mmol) in the presence of potassium hydroxide (0.90g, 16.2mmol) at 0˚C in methanol, as brown solid (0.24g, 79%); R_f= 0.3 (Ethyl acetate/hexane; 4:6), mp = 140-142 ^oC; IR (KBr), ν $_{max}$ in cm^{-1}, 3407, 1643, 1480, 1217, 1007, 768; ^1H NMR (300 MHz, $CDCl_3$) δ 10.40 (s, 1H, NH), 9.11(s, 1H, OH), 7.57 (s, 1H, Ar-H), 7.48 (s, 1H, Ar-H), 7.41-7.39 (d, 1H, *J*= 6.66 Hz, Ar-H), 7.32-7.29 (d, 1H, *J*= 7.98 Hz, Ar-H), 7.07-6.96 (m, 2H, Ar-H), 6.14-6.96 (m, 1H, CH), 5.44-5.39 (d, 1H, *J*= 16.92 Hz, CH_2), 5.32-5.28 (d, 1H, *J*= 10.29 Hz, CH_2) 5.09 (s, 2H, OCH_2), 4.66-4.65 (d, 2H, *J*= 4.41 Hz, OCH_2); ^{13}C NMR (50 MHz,

$CDCl_3$+CCl_4) δ 162.6, 151.9, 150.3, 136.4, 132.9, 132.4, 130.5, 128.9, 128.5, 122.4, 118.4, 118.0, 116.3, 114.5, 69.9, 66.8; ESMS, m/z, 368 $(M+H)^+$; Calculated elemental analysis for $C_{17}H_{15}Cl_2NO_4$: C, 55.45; H, 4.11; N, 3.80, Elemental analysis found: C, 55.48; H, 4.08; N, 3.77.

2-(allyloxy)-5-(4-bromobenzyloxy)-*N*-hydroxybenzamide (18e): It was obtained, by reaction of methyl 2-(allyloxy)-5-(4-bromobenzyloxy)benzoate (0.50g, 1.32mmol) with hydroxylamine hydrochloride (0.92g, 13.2mmol) in the presence of potassium hydroxide (1.48g, 26.4mmol) at 0˚C in methanol, as brown solid (0.39g, 77%); R_f = 0.3 (Ethyl acetate/hexane; 4:6), mp = 95-97 ^oC; IR (KBr), ν $_{max}$ in cm^{-1}, 3398, 1648, 1477, 1213, 1003, 769; ^1H NMR (300 MHz, $CDCl_3$) δ 7.75-7.74 (d, 1H, *J*=3.51 Hz, Ar-H), 7.52-7.50 (d, 2H, *J*= 8.40 Hz, Ar-H), 7.31-7.28 (d, 2H, *J*= 8.85 Hz, Ar-H), 7.15-7.14 (d, 2H, *J*= 2.94 Hz, Ar-H), 7.12-7.11 (d, 2H, *J*= 2.94 Hz, Ar-H), 7.01-6.98 (d, 1H, *J*= 8.85 Hz, Ar-H),6.15-6.01 (m, 1H, CH), 5.54-5.41 (m, 2H, $=CH_2$) 5.03 (s, 2H, OCH_2), 4.76-4.75 (d, 2H, *J*= 5.31, OCH_2); ESMS, m/z, 378 $(M+H)^+$; Calculated elemental analysis for $C_{17}H_{16}BrNO_4$: C, 53.99; H, 4.26; N, 3.70, Elemental analysis found: C, 54.03; H, 4.31; N, 3.66.

Methyl 2-(oxiran-2-ylmethoxy)benzoate (19): It was obtained as colorless syrup (1.0gm, 71%), by reaction of methyl salicylate (1.1gm, 7.0mmol) and epichlorohydrin (3.0ml, 35.0mmol) at room temperature. R_f = 0.48 (Ethyl acetate/hexane; 3:7); IR (neat), ν $_{max}$ in cm^{-1}, 3110, 3021, 2361, 1717, 1216; ^1H NMR (300 MHz, $CDCl_3$) δ 7.82-7.78 (m, 1H, Ar-H), 7.45-7.42 (m, 1H, Ar-H), 7.03-6.97 (m, 2H, Ar-H), 4.35 (dd, J_1 = 2.85Hz, J_2 = 11.1Hz, 1H, OCH_2), 4.12 (dd, J_1 = 4.8Hz, J_2 = 11.1Hz, 1H, OCH_2), 3.89 (s, 3H, OCH_3), 3.40-3.37 (m, 1H, CH), 2.91 (d, *J* = 2.8Hz, 2H, OCH_2); ^{13}C NMR (75 MHz, $CDCl_3$), δ 166.9, 157.9, 134.4, 130.1, 122.7, 120.5, 115.6, 71.2, 52.0, 50.2, 44.2; ESMS, m/z, 209 $(M+H)^+$, Calculated elemental analysis for $C_{11}H_{12}O_4$: C, 63.45; H, 5.81 Elemental analysis found, C, 63.41; H, 5.76.

Methyl 2-(3-azido-2-hydroxypropoxy) benzoate (20): It was obtained as colorless syrup (0.8gm, 67%), by reaction of methyl 2-(oxiran-2-ylmethoxy)benzoate (1.0gm, 4.01mmol) and sodium azide (0.31gm, 4.02mmol) in DMF (10 mL); R_f = 0.45 (Ethyl acetate/hexane; 4:6); IR (neat), ν $_{max}$ in cm^{-1}, 3224, 2932, 2362, 2103, 1254; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90-7.76 (m, 1H, Ar-H), 7.47-7.42 (m, 1H, Ar-H), 7.00-6.95 (m, 2H, Ar-H), 4.09-4.03 (m, 2H, OCH_2), 3.93 (s, 3H, OCH_3), 3.46 (bs, 1H, CH), 3.29 (bs, 1H, OH), 2.96 (s, 1H, NCH_2), 2.84 (s, 1H, NCH_2); ^{13}C NMR (75 MHz, $CDCl_3$), δ 166.7, 159.0, 134.4, 132.1, 121.6, 120.5, 115.2, 71.4, 68.9, 53.1, 52.5; ESMS, m/z, 208 $(M+H)^+$; Calculated Elemental analysis for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.22; N, 16.73 Elemental analysis found, C, 52.50; H, 5.19; N, 16.69.

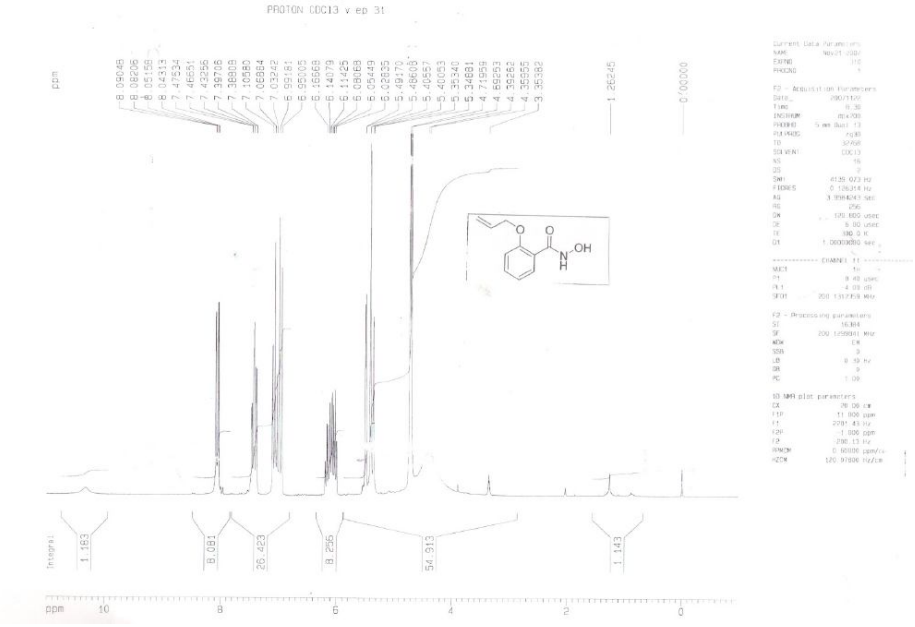
Methyl 2-(2-hydroxy-3-(4-phenyl-*5H*-1,2,3-triazol-1-yl)propoxy)benzoate (21) It was obtained as colorless syrup (0.7gm, 69%), by reaction of methyl 2-(3-azido-2-hydroxypropoxy) benzoate (0.8gm, 3.0mml) and phenyl acetylene (0.32ml, 3.02mmol) in t-BuOH-water-acetone (2:2:1) (6mL) under influence of copper sulphate (10mg 0.3mmol) and sodium ascorbate (26mg, 0.6mmol); R_f= 0.61 (MeOH/$CHCl_3$; 2:8); IR (neat), ν $_{max}$ in cm^{-1}, 3224, 2932, 2362, 1723, 1254; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98-7.97 (m, 1H, Ar-H), 7.81-7.73 (m, 2H, Ar-H), 7.45-7.26 (m, 5H, Ar-H), 7.02-6.93 (m, 2H, Ar-H), 5.0 (bs, 1H, OH) 4.74 (dd, J_1 = 2.3Hz, J_2 = 13.9Hz, 1H, OCH_2), 4.63 (dd, J_1 = 5.7Hz, J_2 = 19.6Hz, 1H, OCH_2), 4.41 (bs, 1H, CH), 4.21 (dd, J_1 = 2.4Hz, J_2 = 9.4 Hz, 1H, NCH_2), 3.89 (s, 4H, NCH_2, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$), δ 171.6, 156.6, 147.0, 134.5, 132.1, 129.1, 128.4, 126.1, 121.9, 121.8, 120.5, 115.2, 71.8, 69.0, 52.7, 52.5; Calculated elemental analysis for $C_{19}H_{19}N_3O_4$: C, 64.58; H, 5.42; N, 11.89 Elemental analysis found, C, 64.52; H, 5.39; N, 11.80.

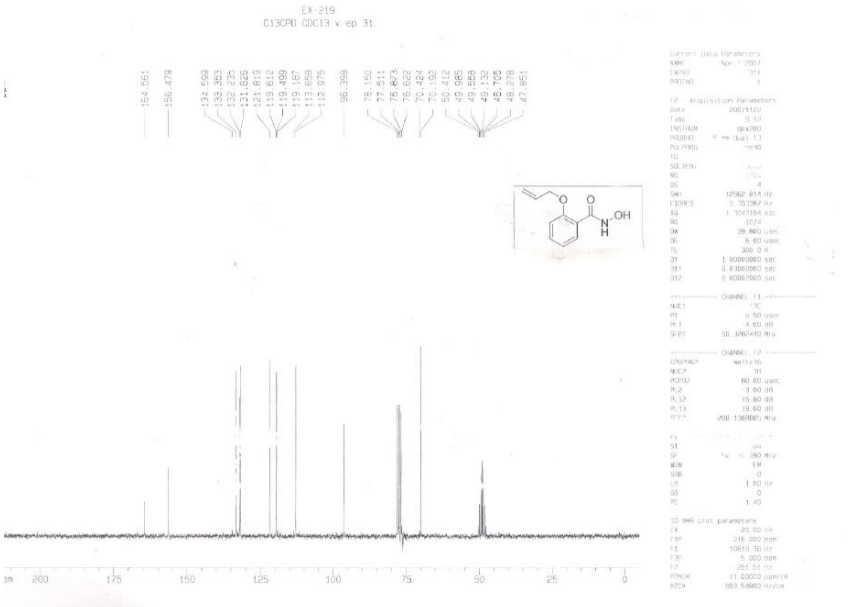
***N*-hydroxy-2-(2-hydroxy-3-(4-phenyl-*5H*-1,2,3-triazol-1-yl)propoxy)benzamide (22)**

It was obtained as white solid (0.49g, 70%), by reaction of 2-(2-hydroxy-3-(4-phenyl-1H-1,2,3-triazol-1-yl)propoxy) benzoate (0.7gm, 1.02mmol) and hydroxylamine hydrochloride (1.3g, 19.02 mmol) in dry methanol at 0 to -5 °C followed by addition of potassium hydroxide (2.2g, 39.0 mmol) at room temperature, R_f = 0.52 (MeOH/$CHCl_3$; 2:8); mp =161-162 ^oC; IR(KBr), ν_{max} in cm^{-1}, 3389, 3019, 2928, 1715, 1604, 1216; ^1H NMR (300 MHz, $CDCl_3$) δ 9.88 (s, 1H, N-OH), 8.49 (s, 1H, N Ar-H), 7.70(s, 1H, CH), 7.02-7.00 (d, J_H=7.2Hz, 1H, Ar-H), 6.79-6.76(m, 1H, Ar-H), 6.64-6.49 (m, 4H, Ar-H), 6.32-6.23 (m, 3H, Ar-H), 5.00 (s, 1H, OH), 3.83-3.68 (m, 1H, OCH_2), 3.51(bs, 1H, CH), 3.28-3.23 (m, 2H, NCH_2); ^{13}C NMR (50 MHz,$CDCl_3$) δ 164.1, 156.8, 147.0, 132.8, 131.6, 130.8, 129.7, 128.7, 126.0, 123.3, 121.9, 114.5, 71.3, 68.5, 53.4; ESMS, m/z, 355 $(M+H)^+$; Calculated elemental analysis for $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81, Elemental analysis found: C, 61.12; H, 5.17; N, 15.79.

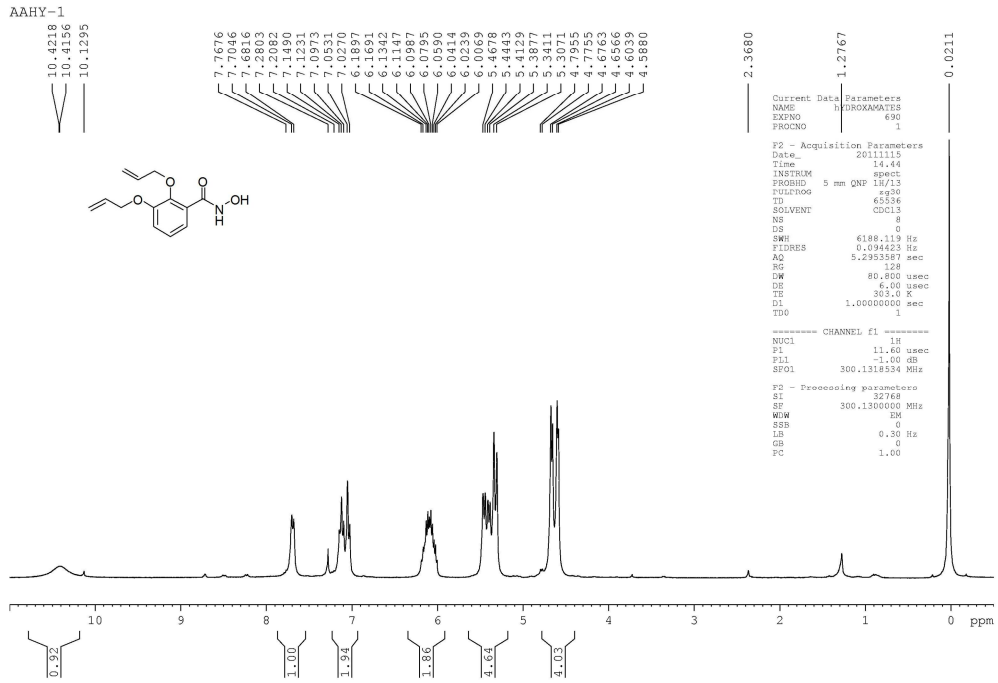
Copies of ^{1}H and ^{13}C NMR spectra of selected compounds:

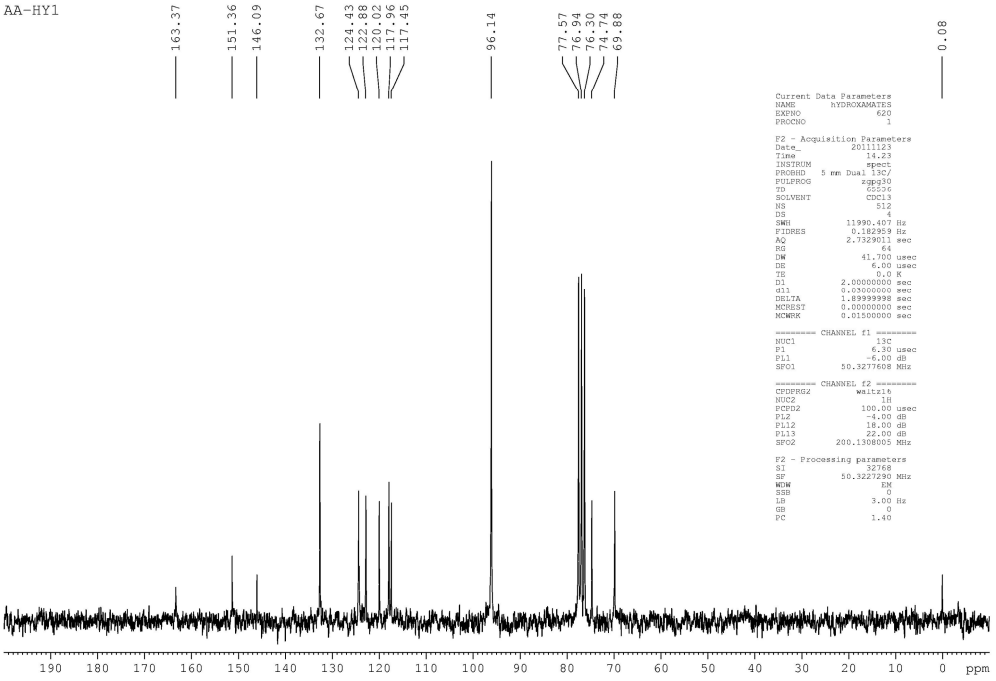
Allyloxy-*N*-hydroxy-benzamide (6a):



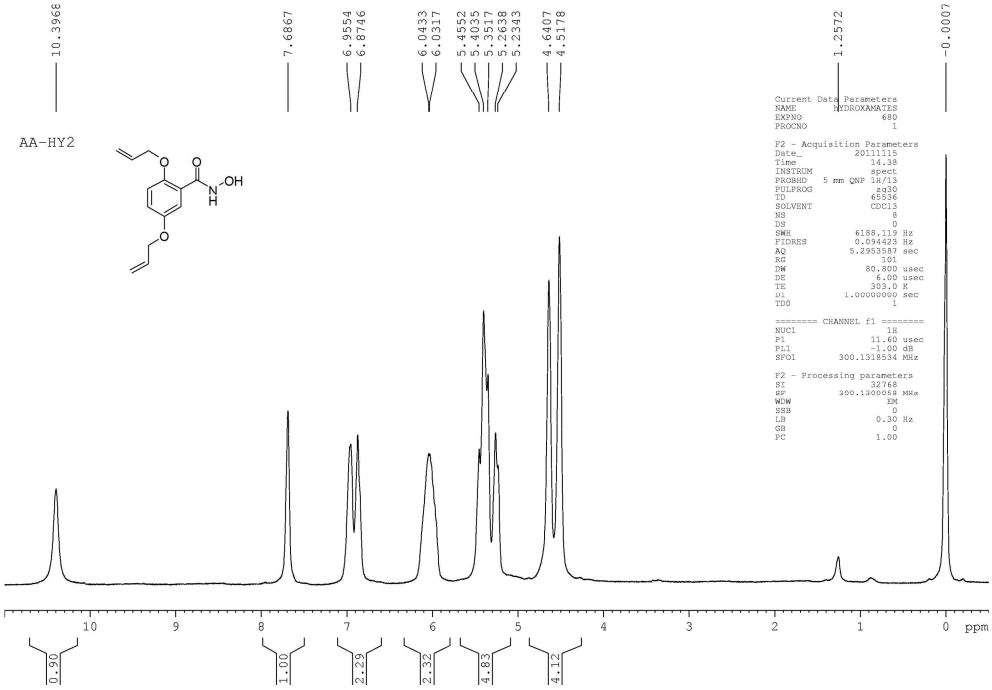


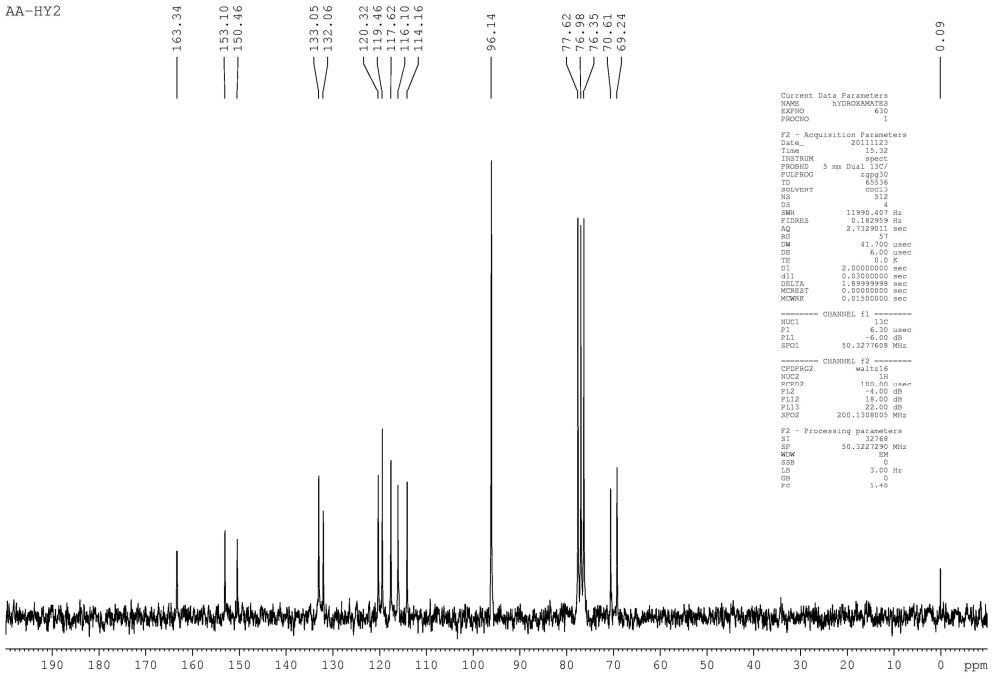
2,3-bis(allyloxy)-*N*-hydroxybenzamide (16a):



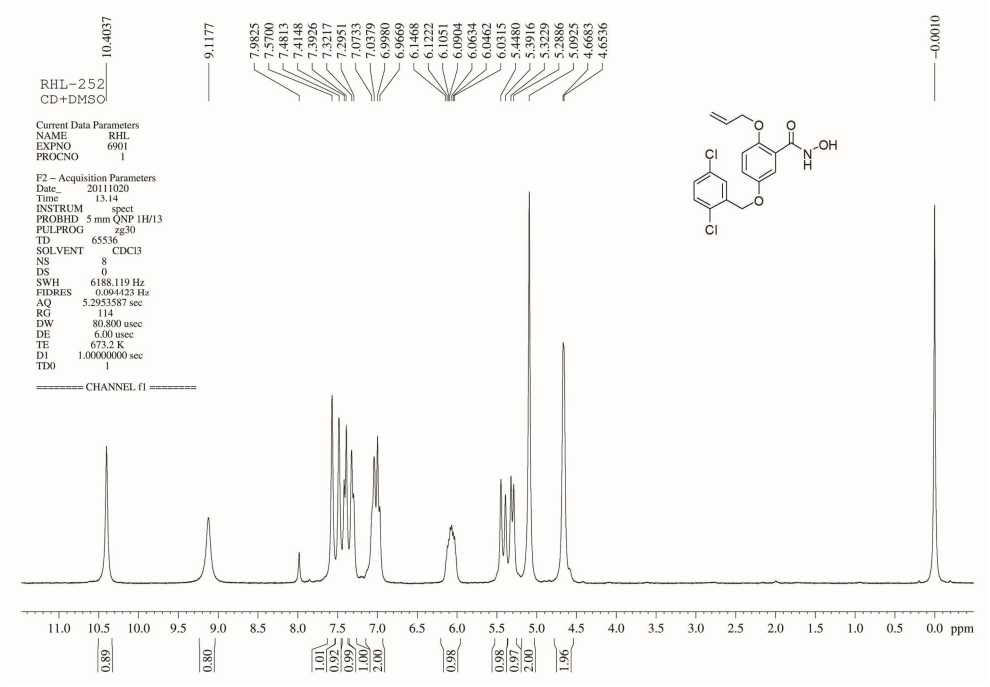


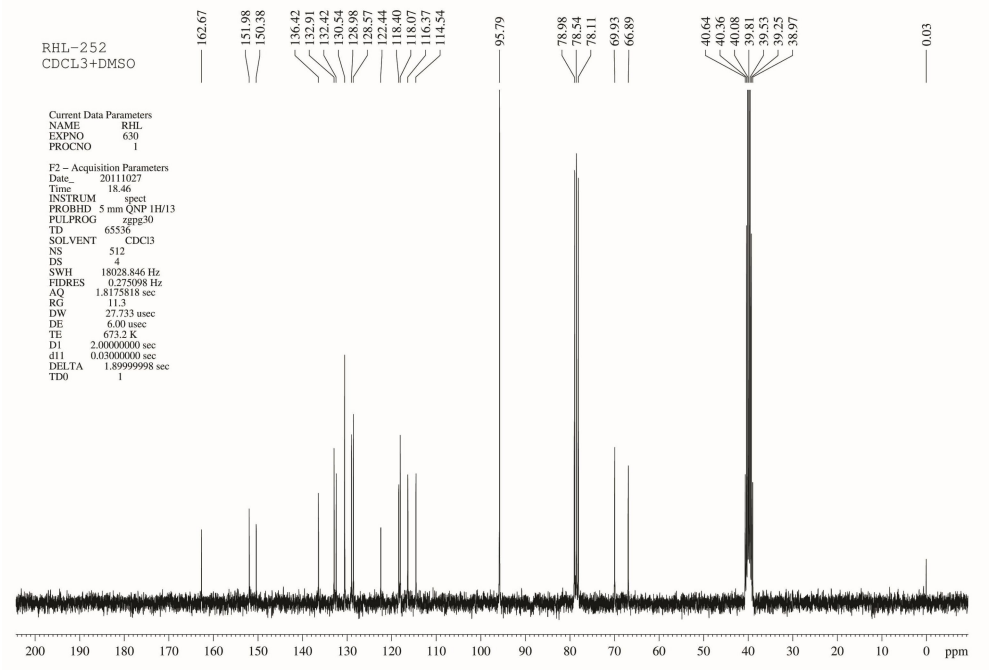
2,5-bis(allyloxy)-*N*-hydroxybenzamide (18a):



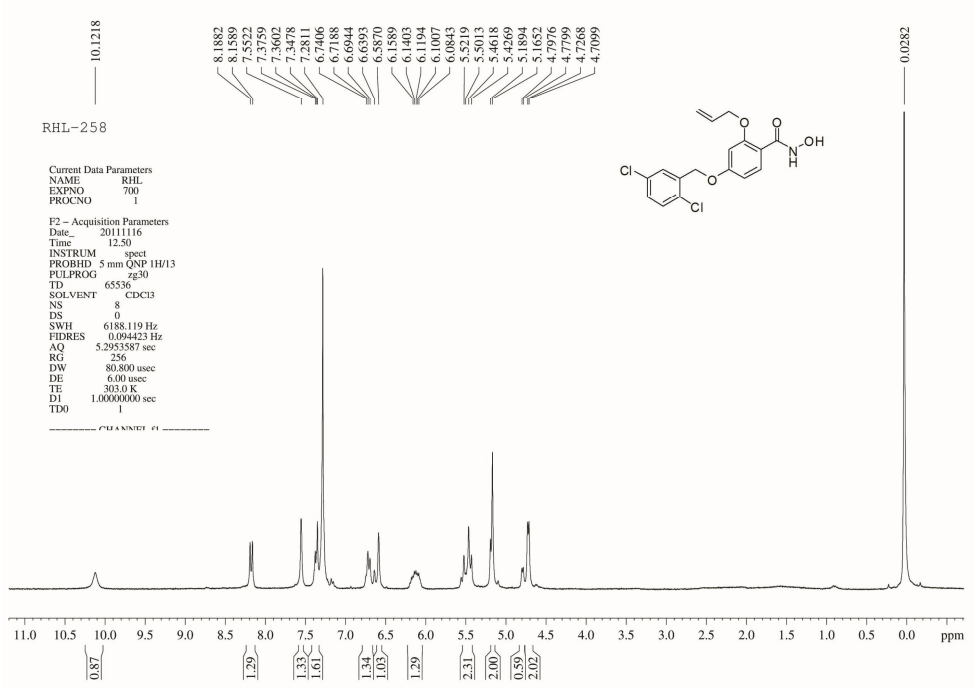


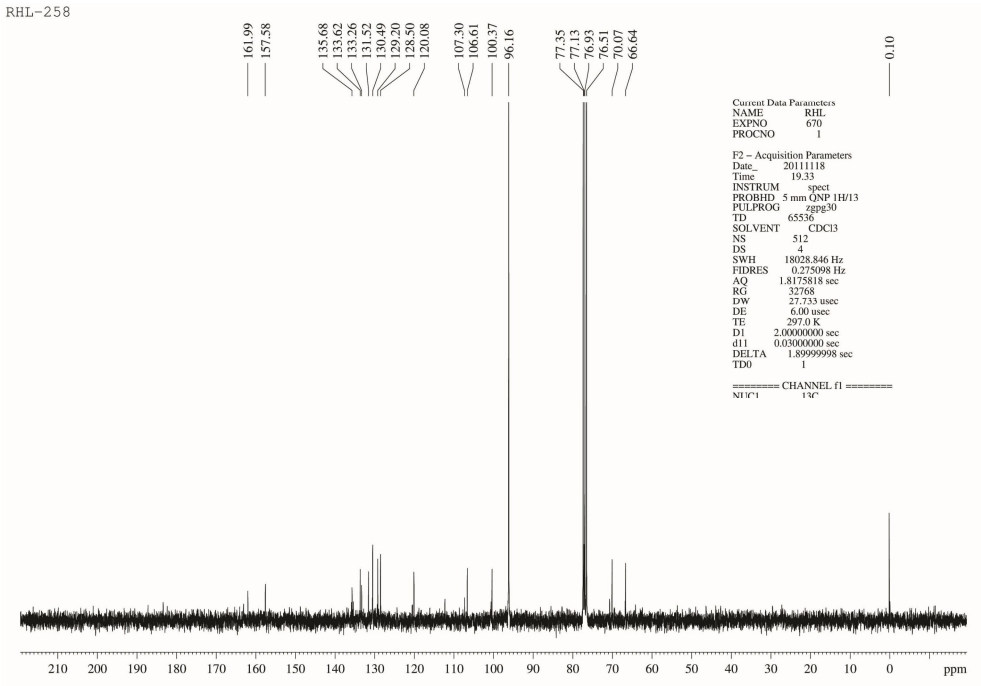
2-(allyloxy)-5-(2, 5-dichlorobenzyloxy)-*N*-hydroxybenzamide (18d):



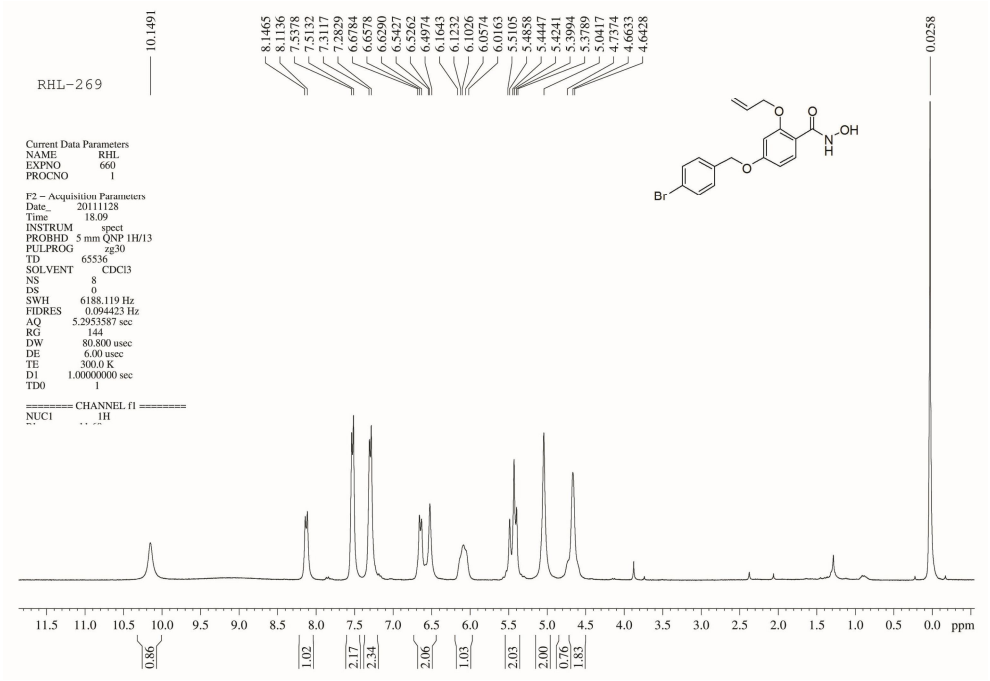


2-(allyloxy)-4-(2, 5-dichlorobenzyloxy)-*N*-hydroxybenzamide (17b):

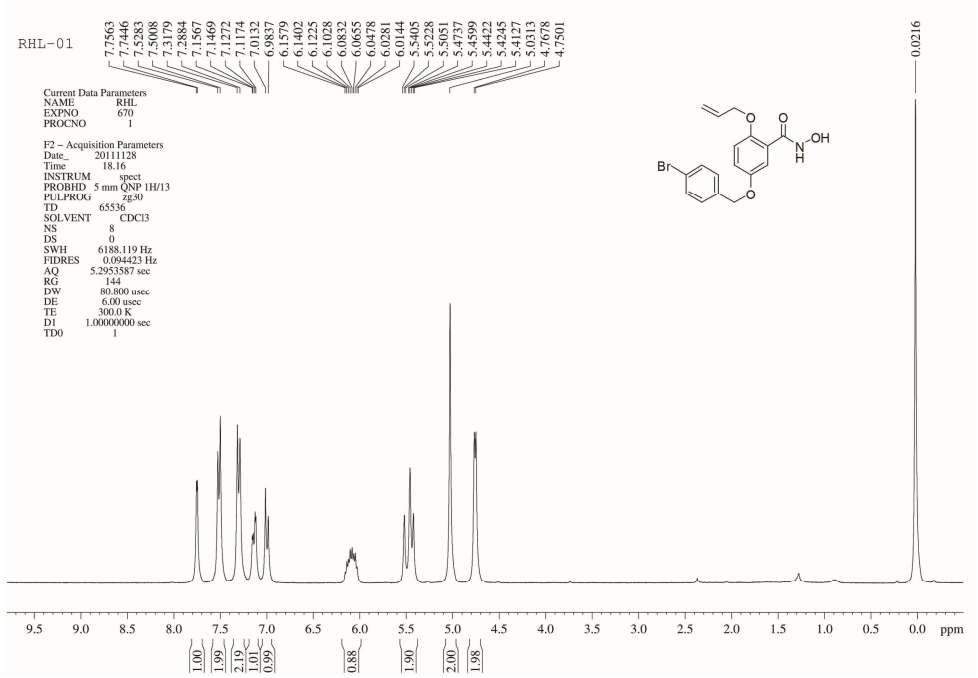




2-(allyloxy)-4-(3-bromobenzyloxy)-*N*-hydroxybenzamide (17c):



2-(allyloxy)-5-(4-bromobenzyloxy)-*N*-hydroxybenzamide (18e):



Electronic Supplementary Material (ESI) for Medicinal Chemistry Communications

Experimental Biology:

Protein purification

The MtuLigA and Human DNA Ligase I were respectively purified after cloning and expression in an *E. coli* based system as reported by us earlier for use in the various assays[1]. The T4 DNA ligase was procured commercially (M/s Sigma).

Molecular Docking

Automated docking calculations were carried out using AutoDock3[2]. A PERL/PerlTk/Python script was used to add to the capability of docking against a ligand database to Autodock3. The structures of the MtuLigA, human DNA ligase I and T4 Ligase were used in the docking calculations using procedures similar to those reported by us earlier[1,3,4].

Ligand Databases

A virtual fragment library FragDB (Fragment Data Base), developed in the laboratory based on available drug-ring systems and functionalities often found in known drug molecules was used as ligand source for virtual screening experiments. The library has been made available by our group to the *Open Source Drug Discovery* Initiative (OSDD) Network, *CSIR* (*http://www.csir.res.in*) and can be accessed at: *http://sysborgtb.osdd.net/bin/view/OpenLabNotebook/FragDBVirtualFragmentLibrary*. The fragments in the library were built and optimized using the Builder module of Insight II (M/S. Accelrys Inc.). Virtual screening studies involving AutoDock3 was used to construct target-specific sets of compounds for *Mtu*LigA. The grid for docking calculations was centered on the active site lysine residue known to interact with the co-factor *viz* Lys123 (PDB: 1ZAU) and a grid size of 60 X 60 X 60 3D affinity grid with 0.375 Å spacing was used for grid map calculations. The Lamarckian genetic algorithm implemented in AutoDock3 was used for all

docking simulations. The binding affinity in terms of docking energies (expressed in kcal/mol) between compounds and the protein were calculated using the scoring function of Autodock3.

DNA end joining assay:

In vitro DNA joining assays for ligase activity were performed using a double-stranded 40 base pair DNA substrate carrying a single-strand nick between bases 22 and 23 as reported earlier (16). Briefly, the substrate was created in Tris EDTA buffer by annealing 22-mer and 18-mer DNA complementary strands to a 40-mer (5'-ATG TCC AGT GAT CCA GCT AAG GTA CGA GTC TAT GTC CAG G-3'). At the 5' end, the 18-mer was radiolabeled with [γ-^{32}P]-ATP (3000 Ci/mmol, Board of Radiation and Isotope Technology, Mumbai). This labeled, nicked 40 bp DNA substrate was used to assay the *in vitro* inhibitory activity of different compounds against *Mtu*LigA, T4Lig and HuLigI. Amounts of the respective enzymes were optimized for similar ligation extents in the absence of any inhibitor under assay conditions.

Full length *Mtu*LigA was cloned expressed and purified as reported earlier.[1,3,4] The assays were carried out using 2 ng of the purified protein. Reaction mixtures (15 µl) containing 50 mM, Tris-HCl, pH 8.0, 5 mM dithiothreitol (DTT), 10 mM $MgCl_2$, 10 % dimethyl sulfoxide (Me_2SO), 50 µM NAD^+, 2 pmol of ^{32}P-labeled nicked duplex DNA substrate and different concentration of compounds were incubated for 1 hr at 25 °C. Subsequently they were quenched with formamide and EDTA. The products were resolved electrophoretically on a 15 % polyacrylamide gel containing 8 M urea in TBE (90 mM Tris-borate and 2.5 mM EDTA). Autoradiograms were developed and ligation extents were measured using Image Master 1D Elite software (Amersham). All compounds were dissolved in 100 % Me_2SO. The compound solutions comprised one-tenth volume of the ligation reaction mixture; thus 10 % Me_2SO was included in all the control reactions.

The same procedure was followed for T4 DNA ligase also. T4 DNA ligase assay was done in a volume of 15 µl containing 0.05 units of enzyme (Amersham), 2 pmol of labeled template, and 66 µM ATP in 66 mM Tris-HCl, pH 7.6, 6.6 mM $MgCl_2$, and 10 mM DTT and 10 % Me_2SO. The Human DNA ligase I expression plasmid was transformed into *E. coli* BL21 (DE3) and purified as described previously[10]. Purified protein was concentrated to 2 mg/ml. 2 µg protein was used for assay in 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, 5 mM DTT, 50 µg/ml BSA and 1 mM ATP as described above.

Calculation of IC_{50} values

The IC_{50} values were determined by plotting the relative ligation activity versus inhibitor concentration and fitting to the equation:

$$V_i/V_0 = IC_{50} / (IC_{50} + [I])$$

using GraphPad Prism®. V_0 and V_i represent rates of ligation in the absence and presence of inhibitor respectively and [I] refers to the inhibitor concentration.

DNA-inhibitor interactions:

Fluorescence assay

DNA intercalating properties of the compounds, if any, were measured by attempting to displace ethidium bromide from DNA. Detection of its displacement from DNA is based on the strong loss in fluorescence that should occur upon its detachment from DNA. The assay mixture contained 5 µg of calf thymus DNA, 5 µM ethidium bromide, 25 mM Tris-HCl, pH 8.0, 50 mM NaCl, and 1 mM EDTA in a total volume of 100 µl. Change in ethidium bromide fluorescence was followed after the addition of increasing inhibitor concentrations at excitation and emission wavelengths of 485 nm and 612 nm respectively.

Gel shift assays

100 ng of plasmid DNA (pUC 18) was incubated with increasing compound concentration in TE at 25 ºC for 1 hr. Subsequently, the DNA was analysed in a 1 % agarose gel similar to procedures reported earlier[1,3,4].

Mode of inhibition of hydroxamates:

Using *Michaelis-Menten* kinetics, saturating substrate concentration for *Mtu*LigA was determined by increasing the NAD^+ concentration from 0.2 μM to 50 μM. K_m for NAD^+ was determined in 10% Me_2SO using the assay procedure. Kinetics for different amount of compounds were determined using varying concentrations of NAD^+ from 0 μM up to 50 μM under standard assay conditions as described earlier.

Rate of the ligation reaction was determined based on the extents of ligation by scanning the gel using Image Master 1D Elite software (Amersham). Data were plotted using *Michaelis-Menten* kinetics in Graph Pad Prism®. Similarly, K_i values were determined by plotting the apparent K_m values against the respective compound concentrations. Mode of inhibition was determined through standard analysis of *Lineweaver-Burk* kinetics.

Determination of the bactericidal activity of compounds:

MIC values for the respective compounds were determined for MtuLigA and T4 DNA ligase A by using two different assay systems. In one assay system we used a temperature sensitive strain of *E. coli* GR501 $ligA^{ts}$. The strain can grow only at 30^0C while its growth is strongly delayed at 37^0C because of a defect in its LigA. This defect is rescued by the expression of *MtuLigA* or *T4 Lig* in it. The latter were cloned into the pTRC99A vector as reported by us earlier[3]. The respective plasmids were transformed into the *E. coli* GR501 $LigA^{ts}$ strain. In growth experiments the strains expressing *MtuLigA* or *T4Lig* were compared with a control GR501 strain carrying empty pTrc99A without any gene insertions at 37ºC. Complementation with

either *Mtu*LigA or T4Lig restores the growth of the mutant strain. Luria–Bertani medium supplemented with 10^5 CFU/ml of *E. coli* $LigA^{ts}$ complemented with Mtu LigA and T4 ligA were incubated with different compound concentration under ambient condition in microtiter plates for 20 hrs and MIC were determined on the basis of the presence of visible growth.

A second assay system involves the *S. typhimurium* LT2 and its DNA ligase minus (null) mutant derivative that had been rescued with a plasmid (pBR313/598/8/1b) encoding the T4Lig gene in order to check the specificity of compounds for NAD^+ -dependent ligases. The native strain harbors its own NAD^+-dependent ligase while the null mutant rescued with the ATP dependent ligase was used to test the relative efficacies of the tested compounds.

References:

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2. G. M. Moriss, D. S. Goodsell, R. S. Halliday, R. Huey, W. E. Hart, R. K. Belew and A. J. Olsan, *J. Comput. Chem.,* 1998, **19**, 1639-1662.
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4. R. P. Tripathi, J. Pandey, V. Kukshal, V. Chaturvedi, A. Ajay, D. Dube, D. Chopra and R. Ramachandran, *Med. Chem. Commun.*, 2011, **2**, 378-384.