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Synthesis of Novel Nitric Oxide Releasing Furoxan Hybrids from Biologically Active Bicyclic Amine and their Antioxidant Activity

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The present paper describes the synthesis of novel nitric oxide hybrids obtained by linking bioactive bicyclic amine to substituted furoxans. The antioxidant activities were studied *in vitro* based on the radical scavenging effect of stable DPPH free radical using ascorbic acid as a standard. The nitric oxide hybrids showed remarkable antioxidant properties and hence, can be employed as potential antioxidant agents.

Keywords: Furoxan, Nitric oxide release assay, Bicyclic amine, Antioxidant activity.

INTRODUCTION

Antioxidants when present even at lower concentrations significantly resists oxidation of substrates which can be oxidized. They can be synthesized *in vivo* or taken as dietary supplements. Antioxidant potential has been attracting a great deal of interest as increased oxidative stress has been established as a major cause in the advancement and development of several potentially fatal diseases, including cardiovascular and neurodegenerative disease. Hence, encouraging methods to counter the undesirable effects of oxidative stress include boosting with either exogenous or endogenous antioxidant defenses of the body [1].

Bicyclic amines display significant application in pharmaceutical field. The octahydropyrrolo pyrimidine derivatives are actively used in the pharmaceutical composition and furthermore for the treatment of neurodegenerative diseases, cardiovascular diseases, inflammatory diseases, autoimmune disorders, cancer, respiratory diseases and fibrosis. It is well established that different derivatives of octahydropyrrolopyrimidine with quinolone moiety show efficacious antimicrobial activity [2]. Octahydro-1*H*-pyrrolo[3,4-*b*]pyridine is an important intermediate in the synthesis of many pharmaceutical ingredients and used for consequent conversion of moxifloxacin as developed by Bayer [3-7]. Keeping in view the biological profile of octahydro-1*H*-pyrrolo[3,4-*b*]pyridine, we coupled it to different furoxans capable of releasing NO to explore their biological activity. Interestingly, furoxan can exist as pair of positional isomers when substituted at different rings. The controversy over its structure, chemistry and the reactivity of its side-chain functional groups have been exhaustively reviewed [8-10]. Many furoxan derivatives display typical NO donor dependent biological activities [11-13]. In fact NO is a physiological messenger involved in a wide range of biological functions [14]. In particular, NO exerts potent effects on vascular homeostasis such as smooth muscle relaxation, inhibition of platelet adherence, aggregation and attenuation of monocyte infiltration. It is identified that NO produced by human white blood cells has toxicological impact on schistosomula [15]. The synthesized compounds were screened for free radical scavenging activity. The parent compounds (**5a-j**) were synthesized as per reported procedure [13].

EXPERIMENTAL

Proton NMR and ^{13}C NMR spectra were recorded on either Bruker Avance (300 MHz), or Varian Inova (400 & 500 MHz) FT spectrometer, internal standard used was tetramethylsilane. Proton NMR spectrum was reported in δ units and coupling constant (*J*) was reported in Hz. HRMS and ESI were recorded on high resolution mass spectrometer (QSTAR-XL). All the chemicals and solvents employed were available commercially.

Column chromatography was performed by using silica gel (60-120 mesh).

Synthesis of compounds (2a-j): To a solution of benzaldehyde (10 mmol) in benzene (20 mL) was added Wittig reagent (15mmol) and refluxed for 10 min with constant stirring. The progress of the reaction was monitored by TLC. After completion of reaction, it was quenched by addition of water. The reaction mixture was extracted with $CHCl_3$ (3 × 15 mL) and concentrated under diminished pressure to yield the crude product, which was further purified using silica gel column chromatography (**Scheme-I**) (Yield: 65-80 %).

Synthesis of compounds (3a-j): Diisobutylaluminum hydride (DIBAL) (11 mmol, 25 %) was added dropwise to the stirred solution ethyl cinnamate (5 mmol) in DCM (15 mL) at 0 ºC. The reaction mixture was allowed to stand at room temperature and continue the stirring for 1 h. After completion of reaction (as monitored by TLC), mixture quenched with saturated solution of sodium potassium tartrate. The organic layer was separated and the aqueous mass was extracted with DCM (3 × 20 mL). The combined organic layer was washed with brine and water, and concentrated using rotary evaporator under reduced pressure and purified by column chromatography technique (**Scheme-I**) (Yield: 60-75 %).

Synthesis of compounds (4a-j): To a stirred solution of compound **3** (10 mmol) in acetic acid (7 mL), aqueous sodium nitrite was added (30 mmol) slowly for 0.5 h. The reaction mixture was stirred at room temperature for 5-9 h. After completion of the reaction (as monitored by TLC), mixture was quenched with ice cold water and extracted with ethyl acetate. The ethyl acetate layer was washed with saturated $NaHCO_3$ and then with water, concentrated using rotary evaporator under reduced pressure and purified by column chromatography technique (**Scheme-I**) (Yield: 30-35 %).

Synthesis of compounds (5a-j): *N*-Bromosuccinimide (NBS) (15 mmol) was added portion wise to a stirred solution of alcohol compound **4** (10 mmol) and PPh_3 (10 mmol) in dichloromethane (20 mL) for 0.5 h and continued the stirring for additional 2 h. After completion of the reaction (as indicated by TLC), the reaction mixture was quenched by addition of water, extracted with dichloromethane (3 × 10 mL) and concentrated under reduced pressure to give the crude product which was purified by silica gel column chromatography (**Scheme-I**) (Yield: 65-75 %).

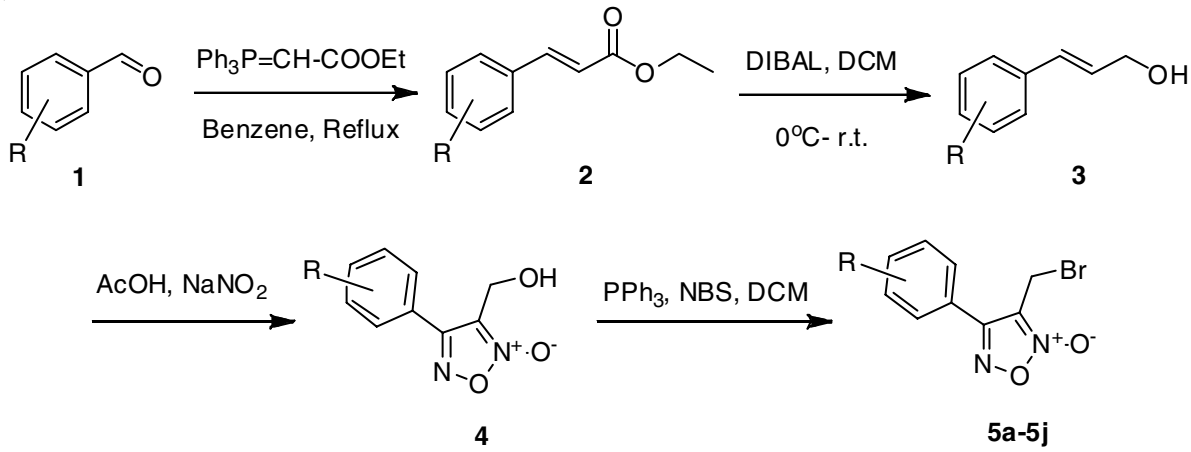
3-(Bromomethyl)-4-(4-nitrophenyl)-1,2,5-oxadiazole-2-oxide (5j): Pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98-7.96 (d, 2H, *J* = 8 Hz), 7.45-7.43 (d, 2H, *J* = 8 Hz), 4.47 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.09, 155.98, 128.25, 118.45, 114.02, 97.01, 15.97; (ESI): ESI-MS (*m/z*): 238 $[M+H]^+$.

Synthesis of compound 7: To a stirred solution of compound **6** (10 mmol) in acetic anhydride (3.5 mL) was heated at 110 ºC for 5 h. The progress of reaction was monitored by TLC, After completion of the reaction (as indicated by TLC), reaction mixture was cooled to room temperature, concentrated under reduced pressure and co-distilled with toluene (5 mL) to give crude compound **7** (yield: 95.6 %) and used for next step without purification **(Scheme-II**).

Synthesis of compound 8: To a stirred solution of compound **7** (10 mmol) in dichloromethane (20 mL) at 0 ºC benzyl amine (15 mmol) was added dropwise. The reaction mixture was stirred for 0.5 h at 90 ºC. After that acetic anhydride (2.5 mL) was added drop wise at 70 ºC and heated to 115 ºC for 4 h. After completion of the reaction (as indicated by TLC), reaction mixture was cooled to room temperature, concentrated under reduced pressure to afford the residue which was recrystallized using ethanol to afford compound **8** (yield: 85.6 %) and used without further purification for the next step (**Scheme-II**).

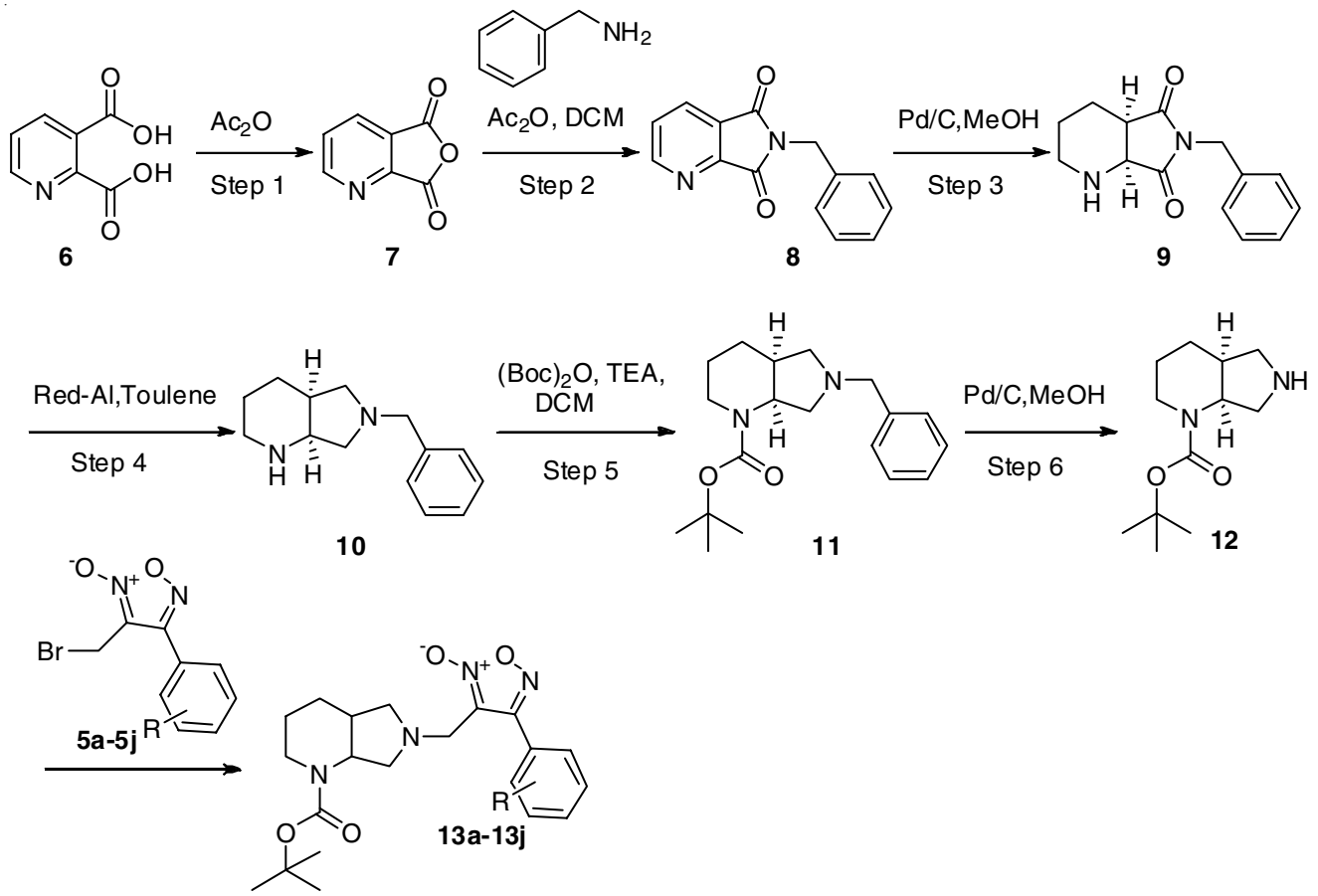
Synthesis of compound 9: To a stirred solution of compound **8** (10 mmol) in methanol (10 mL) at room temperature under nitrogen atmosphere was added 10 % Pd/C (10 mol %). The resulting reaction mixture was hydrogenated under H_2 atmosphere at 60 psi for 4 h, after completion of reaction monitored by TLC, reaction mixture was filtered through celite bed, washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford crude compound **9** (**Scheme-II**) (Yield: 98.1 %).

Synthesis of compound 10: To a stirred solution of compound **9** (20 mmol) in toluene (6 mL) was added 70 % red-Al (70 mmol) at 0 ºC slowly and stirred for 4 h at 67 ºC. After



5a: R = H; **5b:** R = 4-F, **5c:** R = 2-Cl, **5d:** R = 3-OMe, **5e:** R = 2-OMe, **5f:** R = 3-Br, **5g:** R = 2-NO_2, **5h:** R = 4-OMe, **5i:** R = 4-Cl, **5j:** R = 4-NO_2

Scheme-I: Synthetic route of bromo furoxans



Scheme-II: Synthesis of bicyclic NO hybrids

completion of the reaction (as monitored by TLC), reaction mixture was quenched with dilute HCl. Both the organic and aqueous layers were allowed to separate. The aqueous layer contained the desired product in hydrochloride salt form whereas the organic layer was discarded. The aqueous layer was basified by the addition of 50 % NaOH solution to obtain the pH upto 10 and extracted with toluene. The obtained organic layer was washed with brine, dried over anhydrous sodium sulphate and concentrated to afford compound **10** (yield: 39.9 %) as a brown liquid which was used in the next step without purification (**Scheme-II**).

(4a*S*,7a*S*)-6-Benzyloctahydro-1*H*-pyrrolo[3,4-*b*]-pyridine (10): Off white solid; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 7.19-7.3 (m, 5H), 3.95 (br s, 1H), 3.63-3.71 (m, 2H), 3.17-3.20 (m, 1H), 2.76-2.85 (m, 2H), 2.54-2.65 (m, 2H), 2.44 (dd, 2H, J_1 = 10 Hz, J_2 = 2.8 Hz), 2.06-2.11 (m, 1H), 1.43-1.63 (m, 3H) and 1.31-1.37 (m, 1H); ^{13}C NMR (100.57 MHz, $CDCl_3$) δ: 139, 128.5, 128.0, 126.7, 60.4, 59.7, 56.1, 55.1, 43.8, 36.1, 23.7, 21.2; ESI-MS (*m/z*) = 217.1 $[M+H]^+$.

Synthesis of compound 11: To a stirred solution of compound **10** (10 mmol) in dichloromethane (15 mL), triethylamine (TEA) (20 mmol) was added followed by dropwise addition of di-*tert*-butyl dicarbonate (15 mmol) under N_2 atmosphere at 0 ºC. The resulting reaction mixture was allowed to stand to room temperature and stirred for 1 h. After completion of the reaction as monitored by TLC, it was concentrated under reduced pressure to afford crude compound **11** which was purified by column chromatography (**Scheme-II**) (Yield: 80 %).

Synthesis of compound 12: To a stirred solution of compound **11** (10 mmol) in methanol (30 mL) at room temperature under nitrogen atmosphere 10 % Pd/C (10 mol %) was added. The reaction mixture was hydrogenated at 50 ºC and 60 psi for 5 h. After completion of the reaction as monitored by TLC, the reaction mass was filtered through celite bed and was washed with methanol. Obtained filtrate was concentrated under reduced pressure to afford crude compound which was purified by column chromatography (**Scheme-II**) (Yield: 72 %).

(4a*S*,7a*S*)-*tert*-Butyloctahydro-1*H*-pyrrolo[3,4-*b*]pyridine -1-carboxylate (12): ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.31 (d, 1H, J = 7.6 Hz), 3.77 (d, 1H, J = 12.8 Hz), 2.96 (dd, 1H, J_1 = 10.8 Hz, J_2 = 6.0 Hz), 2.85 (dd, 1H, J_1 = 10.4 Hz, J_2 = 8.4 Hz), 2.56-2.67 (m, 3H), 1.88-1.95 (m, 1H), 1.55-1.60 (m, 2H), 1.39 (s, 9H) and 1.23-1.34 (m, 2H); ^{13}C NMR (100.57 MHz, $CDCl_3$) δ: 155.2, 79.6, 54.1, 51.3, 44.5, 39.4, 35.4, 28.4, 25.6, 23.8; ESI-MS (*m/z*) = 227.1 $[M+H]^+$.

Synthesis of substituted nitric oxide releasing derivatives (13a-j): To the stirred solution compound **12** (10 mmol) in DCM, potassium carbonate (22 mmol), compound **5a-j** (10 mmol) was added and stirred at room temperature. After completion of reaction as monitored by TLC, the reaction mass was filtered and solvent was evaporated to obtain the crude product, which was purified by column chromatography (**Scheme-II**). (Yield: 60-70 %).

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-b]pyridin-6(2*H*,7*H*,7a*H*)yl)methyl)-4-phenyl-1,2,5-oxadiazole-2-oxide (13a): White solid; ^{1}H NMR (300 MHz,

$CDCl_3$) δ: 7.66-7.72, (m, 3H), 7.52-7.60 (m, 2H), 3-90-4.04 (m, 2H), 3.85 (s, 2H), 2.87-2.93 (m, 2H), 2.57-2.58 (m, 2H), 2.35-2.41 (m, 1H), 2.03-2.07 (m, 1H), 1.78-1.86 (m, 2H), 1.41 (s, 9H), 1.25-1.33 (m, 2H); ^{13}C NMR (75MHz, $CDCl_3$): δ 157.64, 130.24, 120.41, 117.05, 116.69, 113.80, 113.15, 61.82, 56.98, 55.40, 47.88, 37.41, 29.66, 24.37, 22.71, 19.13; HRMS: Found mass: 401.15106.

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)yl)methyl)-4-(4-fluorophenyl)-1,2,5-oxadiazole-2-oxide (13b): Pale yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.93-8.01 (d, *J* = 9.00 Hz, 2H), 7.34-7.52 (d, *J* = 9.00 Hz, 2H), 3.91-4.69 (m, 2H), 3.89 (s, 2H), 2.92-2.98 (m, 2H), 2.83-2.87 (m, 2H), 2.66-2.69 (m, 1H), 2.15-2.19 (m, 1H), 1.73-1.75 (m, 2H), 1.45 (s, 9H), 1.35-1.39 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 159.99, 147.92, 130.52, 130.24, 126.94, 114.97, 79.78, 58.93, 53.94, 52.87, 47.78, 35.94, 28.92, 26.77, 23.99; ESI-MS (*m/z*): 419 $[M + H]^+$.

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)yl)methyl)-4-(2-chlorophenyl)-1,2,5-oxadiazole 2-oxide (13c): Pale yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.51-7.52 (m, 2H), 7.39-7.42 (m, 2H), 4.15-4.24 (m, 2H), 3.62 (s, 2H), 2.80-2.82 (m, 2H), 2.62-2.64 (m, 2H), 2.17-2.18 (m, 1H), 1.74-1.79 (m, 3H), 1.48 (s, 9H), 1.21-1.28 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.42, 152.21, 130.01, 128.77, 122.44, 113.33, 112.82, 109.41, 108.01, 74.21, 59.12, 53.02, 47.12, 33.05, 29.12, 27.22, 25.31, 23.12, 15.62; ESI-MS (*m/z*): 436 $[M + H]^+$.

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)yl)methyl)-4-(3-methoxyphenyl)-1,2,5-oxadiazole-2-oxide (13d): Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.20 (d, 1H), 7.90-7.92 (m, 1H), 7.65-7.68 (m, 1H), 7.37-7.39 (m, 1H), 3.92-4.74 (m, 2H), 3.85 (s, 3H), 3.75 (s, 2H), 2.87-2.90 (m, 2H), 2.71-2.75 (m, 2H), 2.58-2.60 (m, 1H), 2.18-2.20 (m, 1H), 1.74-1.78 (m, 2H), 1.45 (s, 9H), 1.32-1.37 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 160.02, 130.24, 120.41, 117.05, 116.65, 113.15, 61.82, 56.98, 55.98, 52.82, 48.42, 47.88, 37.41, 29.41, 29.66, 24.33, 22.71; HRMS of $C_{22}H_{31}N_4O_5$ calcd. (found) mass: 431.22890 (431.22870).

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)yl)methyl)-4-(2-methoxyphenyl)-1,2,5-oxadiazole-2-oxide (13e): Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.46-7.53 (m, 2H), 6.98-7.08 (m, 2H), 4.63-4.66 (m, 2H), 3.86 (s, 3H), 3.66-3.78 (m 2H), 2.51-2.56 (m, 2H), 2.34-2.42 (m, 2H), 2.16-2.21 (m, 1H), 1.90-2.11 (m, 1H), 1.62-1.73 (m, 2H), 1.44 (s, 9H), 1.18-1.25 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.41, 155.13, 132.22, 130.20, 120.82, 116.33, 115.06, 110.77, 111.13, 79.52, 58.43, 55.50, 53.60, 48.17, 35.44, 29.62, 28.36, 25.69, 23.22, 17.39; HRMS of $C_{22}H_{31}N_4O_5$ calcd. (found) mass: 431.22890 (431.22870).

4-(3-Bromophenyl)-3-((1-(*tert*-butoxycarbonyl)tetrahydro-1*H*-pyrrolo[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)yl)methyl)-1,2,5-oxadiazole 2-oxide (13f): Pale yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.32 (s, 1H), 7.90-7.93 (d, *J* = 12.00 Hz, 1H), 7.65-7.68 (d, 1H, *J* = 12.00 Hz), 7.37-7.42 (m, 1H), 3.74-4.68 (m, 2H), 3.69 (s, 2H), 2.82-2.85 (m, 2H), 2.63-2.66 (m, 2H), 2.17-2.18 (m, 1H), 1.66-1.77 (m, 3H), 1.45 (s, 9H), 1.21-1.31 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.24, 133.95, 130.65, 128.77, 126.14, 123.11, 112.75, 79.6, 58.37, 53.48, 49.54, 47.53, 35.64, 29.40, 28.42, 26.40, 23.44. HRMS of $C_{21}H_{28}N_4O_4Br_4$ calcd. (found) mass: 479.12884 (479.19220).

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)yl)methyl)-4-(2-nitrophenyl)-1,2,5-oxadiazole-2-oxide (13g): Yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.69-7.71 (m, 2H), 7.52-7.59 (m, 2H), 4.17-4.25 (m, 2H), 3.62 (s, 2H), 2.80-2.83 (m, 2H), 2.63-2.65 (m, 2H), 2.15-2.19 (m, 1H), 1.65-1.77 (m, 3H), 1.45 (s, 9H), 1.20-1.29 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 155.41, 154.13, 130.22, 129.80, 123.12, 115.73, 114.68, 109.79, 108.21, 79.31, 59.43, 53.60, 47.19, 33.89, 29.02, 27.99, 25.32, 23.25, 16.88; ESI-MS (*m/z*): 445 [M].

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)yl)methyl)-4-(4-methoxyphenyl)-1,2,5-oxadiazole-2-oxide (13h): Pale yellow solid; ^{1}H NMR (300 MHz, CDCl3) δ: 7.51-7.54 (d, *J* = 9.00 Hz, 2H), 7.13-7.16 (d, *J* = 9.00 Hz, 2H), 4.52-4.59 (m, 2H), 3.82 (s, 3H), 3.62-3.68 (m 2H), 2.49-2.50 (m, 2H), 2.40-2.41 (m, 2H), 2.14-2.23 (m, 1H), 1.89-2.12 (m, 1H), 1.65-1.72 (m, 2H), 1.43 (s, 9H), 1.19-1.22 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 157.44, 155.22, 132.35, 131.22, 120.92, 116.23, 115.82, 110.62, 111.44, 79.42, 58.33, 55.52, 53.59, 48.16, 35.42, 29.61, 28.35, 25.62, 23.21, 17.30; ESI-MS (*m/z*): 431 $[M+H]^+$.

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)-yl)methyl)-4-(4-chlorophenyl)-1,2,5-oxadiazole-2-oxide (13i): Pale yellow solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.93-7.96 (d, *J* = 9.00 Hz, 2H), 7.52-7.55 (d, *J* = 9.00 Hz, 2H), 3.81-4.71 (m, 2H), 3.69 (s, 2H), 2.83-2.88 (m, 2H), 2.72-2.74 (m, 2H), 2.55-2.58 (m, 1H), 2.16-2.17 (m, 1H), 1.72-1.74 (m, 2H), 1.48 (s, 9H), 1.36-1.38 (m, 2H); 13C NMR (75MHz, CDCl3): 156.37, 137.40, 129.40, 129.25, 125.37, 112.77, 79.74, 58.53, 53.64, 52.46, 47.67, 35.42, 28.42, 26.48, 23.28.

3-((1-(*tert*-Butoxycarbonyl)tetrahydro-1*H*-pyrrolo-[3,4-*b*]pyridin-6(2*H*,7*H*,7a*H*)-yl)methyl)-4-(4-nitrophenyl)-1,2,5-oxadiazole 2-oxide (13j): Yellow solid; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.37-8.39 (d, *J* = 8.00 Hz, 2H,), 8.24-8.26 (d, *J* = 8.00 Hz, 2H), 3.91-4.71 (m, 2H), 3.73 (s, 2H), 2.88-2.90 (m, 2H), 2.72-2.76 (m, 2H), 2.58-2.64 (m, 1H), 2.18-2.20 (m, 1H), 1.74-1.78 (m, 2H), 1.46 (s, 9H), 1.33-1.38 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): 157.30, 155.23, 130.99, 129.02, 127.87, 126.81, 113.11, 79.62, 58.46, 53.63, 47.50, 36.97, 35.37, 29.62, 28.32, 26.39, 23.26; ESI-MS (*m/z*): 446 $[M+H]^+$.

RESULTS AND DISCUSSION

The synthesis of furoxan derivatives (**5a-j**) proceeded in a step-wise manner *via* the reaction of benzaldehydes (**1a-j**) with Witting reagent to yield the corresponding ethyl cinnamates (**2a-j**), followed by reduction with diisobutylaluminum hydride (DIBAL) to form cinnamyl alcohols (**3a-j**), which on further treatment with $AcOH/NaNO_2$ gave furoxan alcohols (**4a-j**). Conversion of alcohols (**4a-j**) to bromo furoxan derivatives (**5a-j**) was effected using *N*-bromosuccinimide (NBS) and triphenyl phosphine (PPh_3) (**Scheme-I**).

The final nitric oxide (NO) hybrids were obtained by coupling bicyclic amine to furoxans (**Scheme-II**). Pyridine-2,3-dicarboxylic acid (compound **6**) was treated with acetic anhydride followed by benzyl amine to afford 6-benzyl-5*H*-pyrrolo[3,4-*b*]-

pyridine-5,7(6*H*)-dione (compound **8**) which was reduced to form (4a*R*,7a*S*)-6-benzyltetrahydro-1*H*-pyrrolo[3,4-*b*]-pyridine-5,7(6*H*,7a*H*)-dione (compound **9**) in the presence of 10 % palladium on carbon in methanol solvent. Compound **9** was purified by acid base treatment and also it could be used without purification. Compound **9** was further reduced in presence of sodium *bis*(2-methoxyethoxy)aluminium hydride (70 % solution in toluene), commercially known as vitride to afford (4a*S*,7a*S*)-6-benzyloctahydro-1*H*-pyrrolo[3,4-*b*]pyridine (compound **10**), then compound **10** was protected with Boc group in TEA and DCM as solvent to afford (4a*S*,7a*S*)-*tert*-butyl-6-benzyloctahydro-1*H*-pyrrolo[3,4-*b*]pyridine-1-carboxylate (compound **11**). Hydrogenolysis (*N*-debenzylation) of compound **11** was carried out using 10 % Pd/C at 60 psi to give the compound *tert*-butyl(4a*S*,7a*S*)-octahydro-1*H*-pyrrolo-[3,4-*b*]pyridine-1-carboxylate (compound **12**). Finally coupling by nucleophilic displacement of bromide furoxans by bicyclic amine (compound **12** afforded the desired novel NO hybrids (compound **13a-j**) (Table-1).

TABLE-1
NO RELEASE DATA AND *in vitro* ANTIOXIDANT ACTIVITY OF COMPOUNDS **13a-j**

Compounds	NO release assay % of NO_2^- mol/mol + L-Cys 5 mM)	Antioxidant activity (%) at different concentrations (µg/mL)		
		10	20	30
13a	14.36 ± 0.41	50.08	62.78	75.56
13b	13.87 ± 0.12	55.15	65.65	80.50
13c	12.96 ± 0.74	52.58	58.96	78.41
13d	14.18 ± 1.0	55.43	70.66	82.00
13e	15.18 ± 0.97	45.97	70.52	80.83
13f	14.27 ± 0.89	48.44	58.32	74.10
13g	15.52 ± 0.56	60.36	75.56	82.32
13h	14.98 ± 0.70	65.28	77.29	85.92
13i	14.72 ± 0.40	51.25	62.99	80.57
13j	15.18 ± 0.35	44.98	56.69	78.05
Propyl nitrite	2.3 ± 0.25	–	–	–
Ascorbic acid	–	83.83	85.79	92.58

Quantitative nitrite detection: The nitric oxide release assay of prepared NO hybrids was carried out using the Griess assay [16]. The ability of hybrid molecules and of corresponding reference compounds to release NO was indirectly evaluated through their capacity to produce nitrite [NO^- 2 % (mol/mol)] at 37 ºC in water solution, at physiological 7.4 pH, in the presence of an extra cysteine (Griess reaction). Nitrite is the most important product of the oxidation of NO in aerobic aqueous solution. The results expressed as NO^- 2 % (mol/mol) are reported in Table-1. The products generate nitrite, in the range of 12% to 16 % in the order **13g > 13e > 13j > 13h > 13i > 13a > 13f > 13d > 13b > 13c**. In particular, reference compound propyl nitrite was able to release NO only to the extent of 2.3% while the bicyclic NO hybrids released NO in the range of 12 to 16 % (Table-1).

Antioxidant activity: The antioxidant activity of prepared NO hybrids of bicyclic amine were based on radical scavenging effect of stable DPPH using ascorbic acid as a standard [13]. From the obtained results (Table-1), it can be seen that compound **13h** was found to exhibit highest antioxidant activity followed by **13g**, **13d**, **13e**, **13i**, **13b**, **13c**, **13j**, **13a** and **13f** showed least antioxidant activity.

Conclusion

In summary, a series of novel nitric oxide releasing derivatives were synthesized by convenient and efficient method. The compounds well characterized by spectroscopic techniques were screened for nitric oxide release assay and antioxidant activity. The synthesized compounds showed considerable antioxidant activity and can be further evaluated for different pharmacological effects.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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