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Synthesis, molecular modelling studies and ADME prediction of benzothiazole clubbed oxadiazole-Mannich bases, and evaluation of their anti-diabetic activity through in vivo model

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ABSTRACT

A small library of new benzothiazole clubbed oxadiazole-Mannich bases (**M-1 to M-22**) were synthesized and characterized by IR, NMR, Mass and Elemental analysis results. Molecular docking studies were done to assess the binding mode and interactions of synthesized hits at binding site of receptor Peroxisome proliferator-activated receptor, PPAR-γ or PPARG (PDB 1FM9). Among the synthesized compounds, nine compounds were selected on the basis of docking score and evaluated for their in vivo anti-diabetic activity using Oral Glucose Tolerance Test (OGTT) in normal rats followed by Streptozotocin (STZ) - induced diabetes. Results indicated that compound **M-14** (161.39 ± 4.38) showed the highest reduction of blood glucose level comparable to that of the standard drug glibenclamide (140.29 ± 1.24) in STZ model. Other compounds exhibited moderate to good anti hyperglycaemic activity. ADME studies was done using Molinspiration online software, revealed that all compounds (except M-11) are likely to be orally active as they obeyed Lipinski's rule of five.

1. Introduction

Several classes of heterocyclic and fused heterocyclic compounds have been identified through molecular biology, empirical screening and rational drug design in search of anti-diabetic agents. Design, synthesis and production of molecules having therapeutic value remain one of the main objectives of bioorganic chemistry. Oxadiazole exists in four possible isomer forms but 1,3,4-oxadiazole is widely explored for various applications [1]. 1,3,4-Oxadiazole heterocycles are reported to possess various pharmacological activities such as antioxidant [2], anticancer [3–5], anti-diabetic [6–8], anti-tubercular [9]. Large number of medicinal compounds used clinically have oxadiazole as pharmacophore e.g. raltagravir an integrase inhibitor, antibacterial furamizole, a PDF inhibitor BB-83698, zibotentan, an anticancer agent, antihypertensive agents tiodazosin and nesapidil, antibiotic furamizole are based on 1,3,4-oxadiazole moiety [10].

Benzo-heterocycle such as Benzothiazole (a phenyl ring fused to a thiazole ring), a weak base is one of privileged heterocycle of interest in medicinal chemistry [11]. Due to the tremendous importance in pharmaceutical applications [12–17], the synthesis of benzothiazole compounds is of considerable interest in chemistry. Some of the medicinal drugs comprising benzothiazole are Riluzole, Sibenadet Hydrochloride (Viozan), Pramipexole [18].

Mannich bases have been reported to possess significant pharmacological activities such as antibacterial, anticancer, antitubercular, and anti-diabetic [19–21].

Diabetes Mellitus (DM) is a metabolic disorder characterized by high levels of blood glucose which might be due to impaired insulin secretion or insulin resistance or both [22]. Type 2 diabetes mellitus (T2DM) or non-insulin dependent diabetes mellitus (NIDDM) is considered as one of the life threatening disease with an increasing occurrence globally [23]. The International Diabetes Federation (IDF) estimated that the global prevalence of diabetes is predicted to grow from 415 million at present to 642 million by 2040 [24]. The Peroxisome proliferator-activated receptors (PPAR's) are ligand activated transcription factors under nuclear receptor superfamily [25]. It is further classified in three subtypes as PPAR-α, PPAR-δ, and PPAR-γ. Among these, PPAR-γ is well known target for the development of anti-hyperglycaemic agents. Thiazolidinediones (TZDs), such as pioglitazone and rosiglitazone, which act on PPAR-γ, are able to improve glucose metabolism and insulin sensitivity, but they have been associated with various

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side effects [26]. The Various synthetic anti-diabetic drugs are also available but many of them are associated with side effects and toxicity [27].

Thus based on the aforementioned points it was thought worthwhile to prepare new Mannich bases having integrated benzothiazole ring and 1,3,4-oxadiazole with an objective to obtain biologically active, safer and relatively low costs anti-diabetic agents. Molecular docking studies are also performed for understanding the binding of ligand to receptor (PPAR-γ or PPARG).

2. Materials and methods

2.1. Chemicals and instruments

All the chemicals used in this work were procured from Merck and Sigma-Aldrich and were used without further purification unless otherwise noted. Progress of the reactions were observed by thin layer chromatography (TLC) using Merck precoated silica gel G plates with a layer thickness 175–225 μm and different eluent systems used were hexane: ethyl acetate: formic acid (5:4:1, v/v/v) and chloroform: methanol (9:1, v/v). The spots of compounds on TLC plates were located by UV irradiation at 254 nm or exposing to iodine vapours. Melting points were measured by the open capillary method with electric melting point apparatus of icon instruments and are uncorrected. Spectroscopic data were measured on the following instruments: ^{1}H NMR and ^{13}C NMR spectra of all the prepared compounds were recorded on a Bruker model DPX 300 MHz and 75 MHz respectively in $CDCl_3$/DMSO d_6; using tetra methyl silane as an internal standard. Chemical shift (δ) values are reported in parts per million (ppm). The splitting pattern abbreviations used are as follows: s for singlet; bs for broad singlet, d for doublet; dd for double doublet; t for triplet; m for multiplet. The IR spectra were recorded on an ATR Bruker spectrophotometer. The FAB mass spectra were recorded using TOF MS ES$^+$ mass spectrometer. Elemental analysis (C, H, N) were performed using Perkin-Elmer 240 analyzer and were found within range of ±0.4% of theoretical values.

2.2. Synthesis

2.2.1. Synthesis of ethyl benzothiazole-2-carboxylate (**1**)

A mixture of o-aminothiophenol (0.1 mol) and diethyl oxalate (0.2 mol) was refluxed for 10 h at 130 °C. After completion of the reaction, the mixture was cooled and poured into a solution containing 50 mL of conc. hydrochloric acid, 150 mL of water and 70 mL of ethanol with stirring, the oil dissolved and a solid formed. After cooling the product was filtered, washed with ethanol, dried overnight and finally recrystallized from ethanol [28]. Yield: 75%; M.p. 69–71 °C, R_f = 0.75. ^{1}H NMR (DMSO d_6, δ, ppm): 1.30–1.35 (3H, t, CH_3 of —$COOCH_2CH_3$); 4.28–4.35 (2H, q, CH_2 of —$COOCH_2$-CH_3); 7.53–8.27 (4H, m, Ar—H). ESI MS (m/z): 208.04 $(M+H)^+$.

2.2.2. Synthesis of benzothiazole-2-carboxyhydrazide (**2**)

The compound (1) (0.01 mol) was dissolved in ethanol (60 mL). To this solution hydrazine hydrate (99%) (0.02 mol) was added drop wise with constant stirring and the mixture was refluxed for 9 h at 80 °C. After completion of reaction mixture was cooled, filtered, washed with water, dried and recrystallized from ethanol [29]. Yield: 85%; M.p. 172–173 °C, R_f = 0.67. ^{1}H NMR ($CDCl_3$, δ, ppm): 4.01–4.25 (2H, s, NH_2 of—$CONHNH_2$); 7.48–8.21 (4H, m, Ar—H); 9.04 (1H, s, NH of —$CONHNH_2$). ESI MS (m/z): 194.3 $(M+H)^+$.

2.2.3. Synthesis of 5-(benzothiazole-2-yl)-1,3,4-oxadiazole-2-thione (**3**)

The compound (2) (0.1 mol) was added to a solution containing 400 mL of 95% ethanol and (0.1 mol) of aqueous solution of KOH (20 mL water). To this reaction mixture carbon disulfide was added and the mixture was refluxed at 80 °C for 13 h. The excess solvent was removed under vacuum using rotary evaporator at 65 °C; the residue thus obtained was dissolved in water and acidified by adding the solution to ice containing HCl (10% to pH ~ 5). The precipitated solid was filtered off, dried and purified by recrystallization from ethanol to give (3) [30]. Yield: 82%; M.p. 145–147 °C, R_f = 0.72. IR (KBr, cm^{-1}); 3423 (N—H), 1210 (C=S). ^{1}H NMR (DMSO d_6, δ, ppm) 7.25 (1H, s, NH); 7.96–8.27 (4H, m, Ar—H). ESI MS (m/z): 236.1 $(M+H)^+$.

2.2.4. General procedure for synthesis of 5-(benzothiazol-2-yl)-3-[substituted amino)methyl]-1,3,4-oxadiazol-2(3H)-thione (**M-1 to M-22**)

To a solution of (3) (0.01 mol) in dioxane and absolute ethanol (1:1, 20 mL), formalin 37% (0.05 mol) was added and the reaction mixture was heated on steam bath till a clear solution was obtained. Appropriate primary or secondary amine (0. 01 mol) in absolute ethanol (5 mL) was added drop wise to the mixture and was stirred overnight at room temperature using parallel synthesizer. After cooling, the formed precipitate was filtered, dried and recrystallized from suitable solvent to give compounds [31].

*2.2.4.1. 5-(Benzothiazol-2-yl)-3-[(4-chlorophenylamino)methyl]-1,3,4-oxadiazol-2(3H)-thione (**M-1**).* Yield: 65%; M.p. 210–211 °C, R_f = 0.65. IR (KBr, cm^{-1}); 3437 (N—H), 2983 (Aliph, C—H), 3329 (Ar, C—H), 1674 (N=C), 1132 (C=S). ^{1}H NMR ($CDCl_3$, δ, ppm): 4.80 (2H, s, N—CH_2—NH); 4.64 (1H, s, NH); 6.98–8.21 (8H, Complex m, Ar—H). ^{13}C NMR (75 MHz, DMSO d_6): δ 57.8, 119.8, 122.5, 122.9, 125.0, 126.5, 129.2, 129.9, 134.0, 140.2, 151.2, 162.4, 163.9, 175.8. ESI MS (m/z): 375 $(M+H)^+$. Anal. Calcd. for $C_{16}H_{11}ClN_4OS_2$: C, 56.06; H, 3.23; N, 16.34; found C, 56.28; H, 3.10; N, 16.01.

*2.2.4.2. 5-(Benzothiazol-2-yl)-3-[(4-fluorophenylamino)methyl]-1,3,4-oxadiazol-2(3H)-thione (**M-2**).* Yield: 70%; M.p. 215–216 °C, R_f = 0.52. IR (KBr, cm^{-1}); 3346 (N—H), 2970 (Aliph, C—H), 3116 (Ar, C—H), 1632 (N=C), 1110 (C=S). ^{1}H NMR ($CDCl_3$, δ, ppm) 4.73 (2H, s, N—CH_2—NH); 4.60 (1H, s, NH); 6.80–7.95 (8H, Complex m, Ar—H). ^{13}C NMR (75 MHz, DMSO d_6): δ 56.6, 117.0, 118.7, 121.8, 122.0, 126.2, 127.0, 135.4, 139.7, 149.8, 160.5, 162.8, 164.2, 174.9. ESI MS (m/z): 359 $(M+H)^+$. Anal. Calcd. for $C_{16}H_{11}FN_4OS_2$: C, 53.62; H, 3.09; N, 15.63; found C, 53.45; H, 3.39; N, 15.32.

*2.2.4.3. 5-(Benzothiazol-2-yl)-3-[(4-nitrophenylamino)methyl]-1,3,4-oxadiazol-2(3H)-thione (**M-3**).* Yield: 68%; M.p. 208–209 °C, R_f = 0.73. IR (KBr, cm^{-1}); 3420 (N—H), 2958 (Aliph, C—H), 3130 (Ar, C—H), 1615 (N=C), 1126 (C=S). ^{1}H NMR ($CDCl_3$, δ, ppm) 4.60 (2H, s, N—CH_2—NH); 5.12 (1H, s, NH); 6.64–8.21 (8H, Complex m, Ar—H). ^{13}C NMR (75 MHz, DMSO d_6): δ 58.0, 114.8, 123.1, 123.6, 124.6, 125.8, 127.9, 134.9, 140.7, 143.3, 151.5, 161.6, 163.4, 176.2. ESI MS (m/z): 386 $(M+H)^+$. Anal. Calcd. for $C_{16}H_{11}N_5$-O_3S_2: C, 49.86; H, 2.88; N, 18.17; found C, 49.65; H, 2.59; N, 18.30.

*2.2.4.4. 5-(Benzothiazol-2-yl)-3-[(2-fluorophenylamino)methyl]-1,3,4-oxadiazol-2(3H)-thione (**M-4**).* Yield: 65%; M.p. 216–217 °C, R_f = 0.63. IR (KBr, cm^{-1}); 3145 (N—H), 2952 (Aliph, C—H), 3125 (Ar, C—H), 1623 (N=C), 1133 (C=S). ^{1}H NMR ($CDCl_3$, δ, ppm) 4.48 (2H, s, N—CH_2—NH); 4.77 (1H, s, NH); 6.61–8.10 (8H, Complex m, Ar—H). ^{13}C NMR (75 MHz, DMSO d_6): δ 57.4, 115.7, 121.2, 122.7, 124.0, 124.1, 124.9, 126.6, 126.8, 135.1, 139.8, 149.9, 152.9, 162.6, 164.4, 174.4. ESI MS (m/z): 359 $(M+H)^+$. Anal. Calcd. for $C_{16}H_{11}FN_4OS_2$: C, 53.62; H, 3.09; N, 15.63; found C, 53.73; H, 3.28; N, 15.44.

*2.2.4.5. 5-(Benzothiazol-2-yl)-3-[(4-bromophenylamino)methyl]-1,3,4-oxadiazol-2(3H)-thione (**M-5**).* Yield: 72%; M.p. 220–222 °C, R_f = 0.76. IR (KBr, cm^{-1}); 3139 (N—H), 2961 (Aliph, C—H), 3118