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Comparative NMR Spectral and Pharmacological Investigation of Some N^1-(4-Substituted benzyl/butyl)-2-methyl-4-nitro-1*H*-imidazoles

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One of the key advantages of NMR spectroscopy is its non-destructive nature, allowing researchers to analyze organic compounds without altering or damaging the samples. This characteristic makes NMR spectroscopy particularly well-suited for studying delicate or limited-quantity samples, providing researchers with a non-invasive means of exploring the molecular intricacies of organic compounds. The effect of substituents in benzyl moiety of N^1-(4-substituted benzyl)-2-methyl-4-nitro-1*H*-imidazoles (**3a-f**: 4-R-C_6H_4-CH_2-N^1; **3a**: C_6H_5-CH_2; **3b**: 4-Br-C_6H_5-CH_2; **3c**: 4-Cl-C_6H_5-CH_2; **3d**: 4-F-C_6H_5-CH_2; **3e**: 4-CH_3-C_6H_5-CH_2 and **3f**: 4-NO_2-C_6H_5-CH_2) and the effect of substituents in N^1-butyl-2-methyl-4-nitro-1*H*-imidazoles (**3g-i**: **3g**: *n*-C_4H_9-, **3h**: *sec*-C_4H_9- and **3i**: *iso*-C_4H_9-) were investigated based on the comparative study of the effect on their chemical shift values with nine different substituents by using ^{1}H NMR and ^{13}C NMR spectra. This work deals with the changes in the chemical shift values with respect to an active methylene carbon (4-R-C_6H_4-CH_2-N^1) and aromatic carbons. The 4-nitrobenzyl moiety of compound **3f** shown more effective deviation on its chemical shift values of the *N*-methylene as well as aromatic protons compared to other substituents in the six imidazoles in ^{1}H and ^{13}C NMR spectra. The effective deviation on its chemical shift values of **3g-i** with respect to decreases the carbon chain length of the butyl groups. Compounds **3a-i** were also screened for their anti-inflammatory and antidiabetic activity at different concentrations (20, 40, 80, 200 and 400 µg/mL). Diclofenac sodium and acarbose were used as standard drugs for anti-inflammatory and antidiabetic activities, respectively. The 4-nitro-1*H*-imidazoles **(3a-i)** showed good to remarkable anti-inflammatory and good to excellent antidiabetic activities compared to their corresponding standard drug.

Keywords: Substituted 4-nitroimidazoles, NMR spectroscopy, Anti-inflammatory activity, Antidiabetic activity.

INTRODUCTION

For researchers working in synthetic chemistry and its allied fields, nuclear magnetic resonance (NMR) spectroscopy is a vital analytical technique. The composition of the samples and their purity could also be ascertained, in addition to the molecular structure [1-4]. Several organic syntheses produce nitroimidazoles as crucial intermediates. Several nitroimidazole derivatives can exhibit varied pharmacological effects as well as other fine chemicals depending on the type of substituents, the position of the nitro group and *N*-alkylated nitroimidazoles as valuable structural motifs [5-10]. Numerous protozoan and

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bacterial infections in humans and animals are frequently treated with nitroimidazoles including metronidazole, misonidazole, ornidazole, secnidazole, etanidazole and tinidazole [11-13].

In the framework of our interest, a comparison studies on the effects of the substituents in benzyl moieties on the chemical shift values at fourth position of the substituted 4-nitro-1*H*-imidazoles (**3a-f**) were conducted using NMR spectral data. The effect of three different butyl moieties on the chemical shift values of the N^1-butyl-2-methyl-4-nitro-1*H*-imidazoles (**3g-i**) were also investigated. Moreover, the potency of anti-inflammatory and antidiabetic activities of N^1-(4-substituted benzyl)-2-methyl-4-nitro-1*H*-imidazoles (**3a-f**) were also evaluated.

EXPERIMENTAL

N^1-(4-Substitutedbenzyl)-2-methyl-4-nitroimidazoles (**3a-f**: 4-R-C_6H_4-CH_2-N^1. **3a**: C_6H_5-CH_2; **3b**: 4-Br-C_6H_5-CH_2; **3c**: 4-Cl-C_6H_5-CH_2; **3d**: 4-F-C_6H_5-CH_2; **3e**: 4-CH_3-C_6H_5-CH_2 and **3f**: 4-NO_2-C_6H_5-CH_2. Fig. 1) N^1-butyl-2-methyl-4-nitro-1*H*-imidazoles (**3g-i: 3g**: *n*-C_4H_9-, **3h:** *sec*-C_4H_9- and **3i:** *iso*-C_4H_9-) were synthesized and purified according to the procedure reported in the literature [14,15]. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DRX 500 NMR spectrometer (400 or 500 MHz) using $CDCl_3$ or DMSO-d_6 as solvent.

Anti-inflammatory assay (BSA denaturation method): N^1-(4-Substituted benzyl/butyl)-2-methyl-4-nitroimidazoles (**3a-i**, Fig. 1) were screened for their anti-inflammatory activity at five different concentrations (20, 40, 80, 200 and 400 µg/mL) by using bovine serum albumin (BSA) denaturation method with minor modifications of the described procedure [15-17]. Briefly, compounds **3a-i** and standard drug were dissolved in minimum quantity of DMF and diluted with phosphate buffer (0.2 M, pH 7.4). The final concentration of DMF in all solution was less than 2.5%. Test solution (2.5 mL) containing different concentrations of drug was mixed with 1 mL of 1 mM bovine serum albumin solution in phosphate buffer and incubated at 37 ºC in an incubator for 10 min. Denaturation was induced by keeping the reaction mixture at 70 ºC in a water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Percentage of inhibition of denaturation was calculated from control where no drug was added. The percentage inhibition of denaturation was calculated by using eqn. 1 [15-17]:

$$\text{Inhibition (\%)} = \frac{A_c - A_t}{A_c} \times 100 \quad (1)$$

where A_c is absorbance of control and A_t is absorbance of test.

Antidiabetic assay (α-amylase inhibition method): N^1-(4-Substituted benzyl/butyl)-2-methyl-4-nitroimidazoles (**3a-i**, Fig. 1) were screened for their antidiabetic activity at five different concentrations (20, 40, 80, 200 and 400 µg/mL) by using the α-amylase inhibition method with minor modifications of reported procedure [15-17]. Briefly, α-amylase (0.2%) was incubated with and without samples (1.5 mL) and standard for 10 min at 25 ºC. This experiment was performed in 0.2 M phosphate buffer (pH 6.9). After pre-incubation, 1% starch solution (0.5 mL) was added and the reaction mixture was incubated for 30 min at 25 ºC. In order to stop the enzymatic reaction, DNSA reagent (0.5 mL) was added as colour reagent and then incubated in a boiling water bath for 90 min. After cooling down to the room temperature, 0.5 mL of samples were diluted to 2.5 mL of distilled water and the absorbance measured at 540 nm using a UV-visible spectrophotometer. The measured absorbance was compared with that of the control experiment. The percentage inhibition was calculated from the given formula [15-17].

$$\text{Inhibition (\%)} = \frac{A_c - A_t}{A_c} \times 100 \quad (2)$$

where A_c is absorbance of control and A_t is absorbance of test.

RESULTS AND DISCUSSION

Comparative ^{1}H NMR spectral studies: The ^{1}H NMR spectrum of 1-benzyl-2-methyl-4-nitro-1*H*-imidazole (**3a**) showed a singlet peaks at δ 7.65 ppm and δ 2.39 ppm (Fig. 2), which are due to aromatic C-H proton (C**H**$_{\text{imidazole ring}}$) and methyl protons (Im-C**H**$_3$) of 4-nitroimidazole ring, respectively. The characteristic peak for methylene protons of *N*-benzyl ring (*N*-CH_2-Ar in **3a**) was obtained at δ 5.09 ppm as singlet peak (Fig. 2), which confirms the formation of *N*-benzylation of 2-methyl-4(5)-nitroimidazole (**1a**). The aromatic protons of phenyl ring (-C**H**$_{\text{phenyl ring}}$) showed a multiplet at δ 7.39-7.37 ppm for three protons (-C**H**$^{3,4,5}_{\text{phenyl ring}}$) and a doublet of doublet for two protons (-C**H**$^{2,6}_{\text{phenyl ring}}$) 7.14-7.12 ppm.

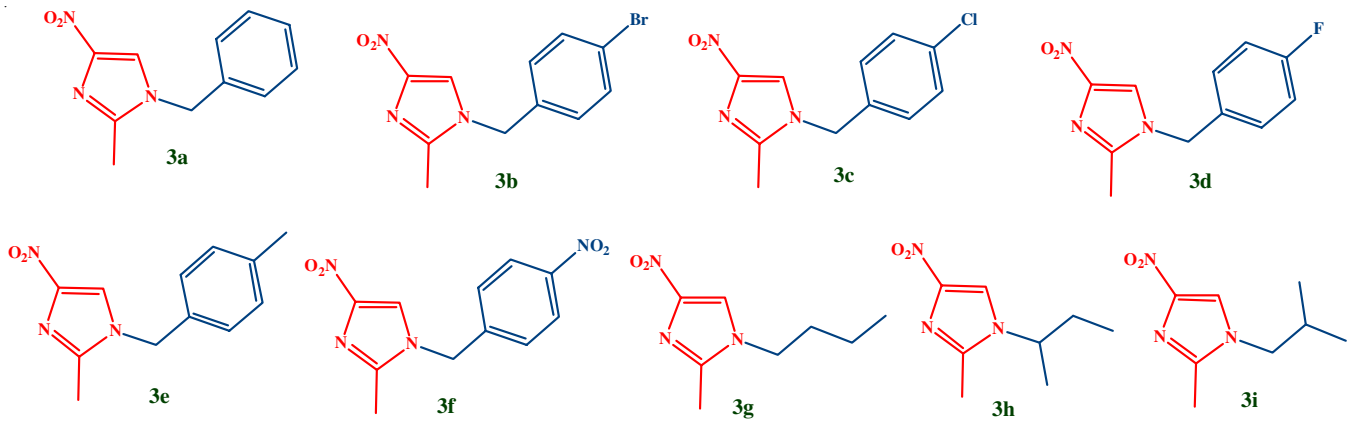

Fig. 1. N^1-(4-Substitutedbenzyl/butyl)-2-methyl-4-nitroimidazoles [Ref. 14,15]

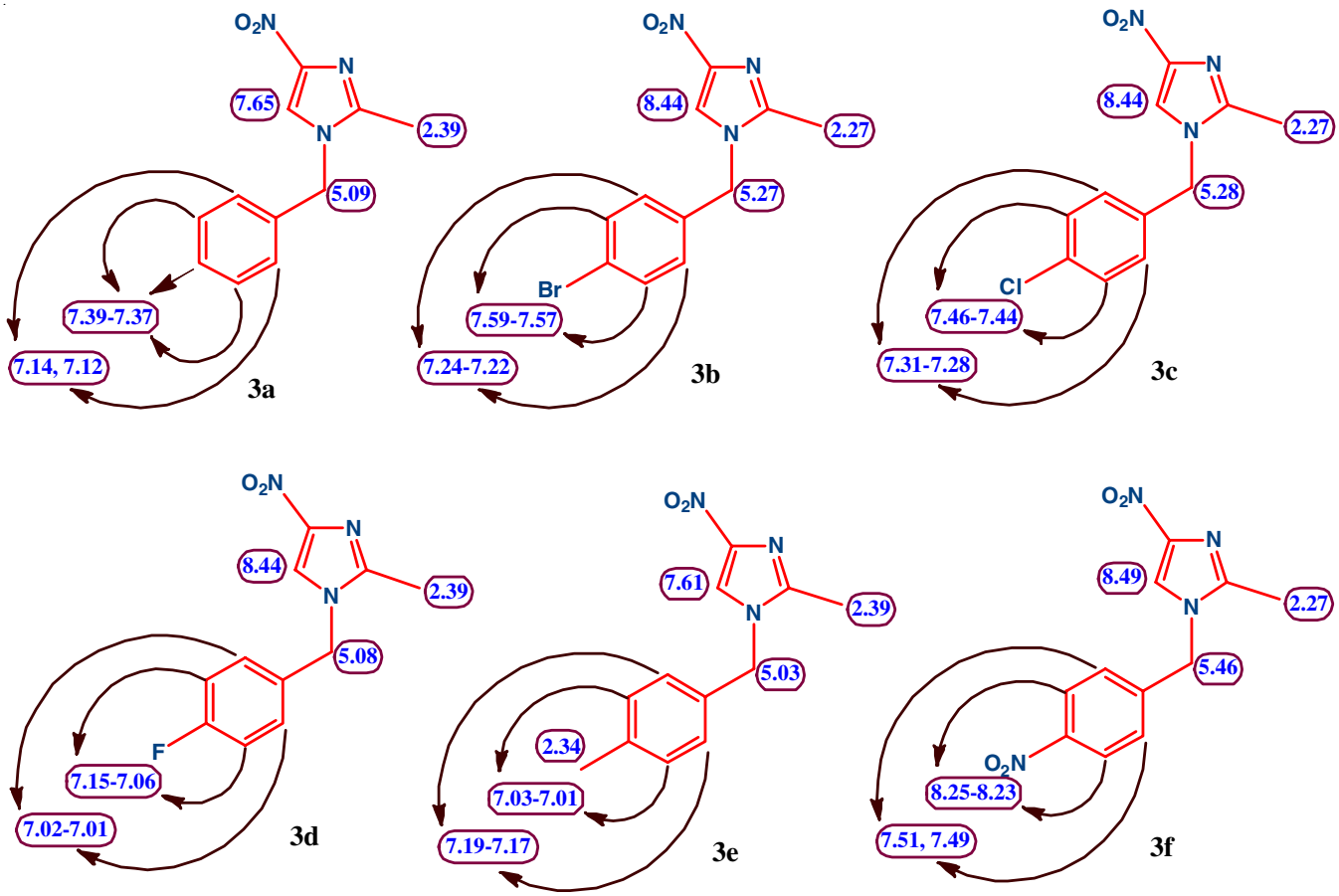

Fig. 2. ^{1}H NMR spectral representation of **3a-f**

The ^{1}H NMR spectrum of 1-(4-bromobenzyl)-2-methyl-4-nitro-1*H*-imidazole (**3b**) showed as a singlet peak at δ 8.44 ppm (4-NO_2-isomer) and as singlet peak at δ 2.27 ppm, which are due to aromatic C-H proton (C**H**$_{\text{imidazole ring}}$) and methyl protons (Im-C**H**$_3$) of 4-nitroimidazole ring, respectively. The characteristic peak for methylene protons of N-benzyl ring (*N*-C**H**$_2$-Ar in **3b**) appeared at δ 5.27 ppm as singlet peak (Fig. 2), which confirms the formation of *N*-(4-bromobenzylation) of 2-methyl-4(5)-nitroimidazole (**1a**). The aromatic protons of phenyl ring (-C**H**$_{\text{phenyl ring}}$) showed at δ 7.59-7.57 ppm as multiplet for two protons and 7.24-7.22 ppm as a multiplet for two protons.

The ^{1}H NMR spectrum of 1-(4-chlorobenzyl)-2-methyl-4-nitro-1*H*-imidazole (**3c**) showed as a singlet peak at δ 8.44 ppm (4-NO_2-isomer) and as singlet peak at δ 2.27 ppm, which are due to aromatic C-H proton (C**H**$_{\text{Imidazole ring}}$) and methyl protons (Im-C**H**$_3$) of 4-nitroimidazole ring, respectively. The characteristic peak for methylene protons of *N*-benzyl ring (N-CH_2-Ar in **3c**) appeared at δ 5.28 ppm as singlet peak (Fig. 2), which confirms the formation of *N*-(4-chlorobenzylation) of 2-methyl-4(5)-nitroimidazole (**1a**). The aromatic protons of phenyl ring showed at δ 7.46-7.44 ppm as multiplet for two protons (-C**H**$^{3,5}_{\text{phenyl ring}}$) and δ 7.31-7.28 ppm as multiplet for two protons (-$CH^{2,6}_{\text{phenyl ring}}$).

The ^{1}H NMR spectrum of 1-(4-fluorobenzyl)-2-methyl-4-nitro-1*H*-imidazole (**3d**) showed as a singlet peak at δ 7.64 ppm (4-NO_2-isomer) and as singlet peak at δ 2.39 ppm, which are due to aromatic C-H proton (C**H**$_{\text{Imidazole ring}}$) and methyl protons (Im-C**H**$_3$) of 4-nitroimidazole ring, respectively. The characteristic peak for methylene protons of *N*-benzyl ring (*N*-CH_2-Ar in **3d**) appeared at δ 5.08 and 5.53 ppm as singlet peak (Fig. 2), which confirms the formation of *N*-(4-fluorobenzylation) of 2-methyl-4(5)-nitroimidazole (**1a**). The aromatic protons of phenyl ring showed at δ 7.15-7.06 ppm as multiplet for two protons (-C**H**$^{3,5}_{\text{phenyl ring}}$) and 7.02-7.01 ppm as multiplet for two protons (-C**H**$^{2,6}_{\text{phenyl ring}}$).

The ^{1}H NMR spectrum of 2-methyl-1-(4-methylbenzyl)-4-nitro-1*H*-imidazole (**3e**) showed as a singlet peak at δ 7.61 ppm (4-NO_2-isomer) and as singlet peak at δ 2.34 ppm, which are due to aromatic C-H proton (C**H**$_{\text{Imidazole ring}}$) and methyl protons (Im-C**H**$_3$) of 4-nitroimidazole ring, respectively. The characteristic peak for methylene protons of N-benzyl ring (*N*-C**H**$_2$-Ar in **3d**) appeared at δ 5.03 ppm as singlet peak (Fig. 2), which confirms the formation of *N*-(4-methylbenzylation) of 2-methyl-4(5)-nitroimidazole (**1a**). The aromatic protons of phenyl ring showed at δ 7.03-7.01 ppm as multiplet for two protons (-C**H**$^{3,5}_{\text{phenyl ring}}$) and δ 7.19-7.17 ppm as doublet for two protons (-C**H**$^{2,6}_{\text{phenyl ring}}$). The chemical shift of methyl protons of phenyl ring (Ph**C**$_4$$^-$$CH_3$) is shown at δ 2.39 ppm as a singlet peak.

The ^{1}H NMR spectrum of 2-methyl-4-nitro-1-(4-nitrobenzyl)-1*H*-imidazole (**3f**) showed as a singlet peak at δ 8.49 ppm (4-NO_2-isomer) and as singlet peak at δ 2.27 ppm, which are due to aromatic C-H proton (C**H**$_{\text{Imidazole ring}}$) and methyl protons (Im-C**H**$_3$) of 4-nitroimidazole ring, respectively. The characteristic peak for methylene protons of N-benzyl ring (*N*-C**H**$_2$-Ar

in **3f**) appeared at δ 5.46 ppm as singlet peak (Fig. 2), which confirms the formation of *N*-(4-nitroobenzylation) of 2-methyl-4(5)-nitroimidazole (**1a**). The aromatic protons of phenyl ring showed at δ 8.25-8.23 ppm as multiplet peak for two protons (-C$\mathbf{H}^{3,5}_{\text{phenyl ring}}$) and 7.51-7.49 ppm as doublet for two protons (-C$\mathbf{H}^{2,6}_{\text{phenyl ring}}$).

In comparison, the chemical shift value of methylene protons (*N*-C$\mathbf{H}_2$-C_6H_4-R) is shown at δ 5.09 ppm (Fig. 2) for unsubstituted benzylated nitroimidazole (C_6H_5-CH_2-*N*, **3a**). The chemical shift obtained at δ 5.27 ppm is due to bromo group substituted in the 4th position of benzyl moiety of nitroimidazole (4-Br-C_6H_4-CH_2-*N*, **3b**). The chemical shift observed at δ 5.28 ppm, which is due to chloro group substituted in the 4th position of a benzyl moiety of nitroimidazole (4-Cl-C_6H_4-CH_2-*N*, **3c**). The chemical shifts appeared at δ 5.08, 5.53 ppm, which are due to fluoro group substituted in the 4th position of a benzyl moiety of nitroimidazole (4-F-C_6H_4-CH_2-*N*, **3d**), whereas the chemical shift appeared at δ 5.03 ppm is due to chloro group substituted in the 4th position of a benzyl moiety of nitroimidazole (4-CH_3-C_6H_4-CH_2-*N*, **3e**). In case of compound **3f**, the chemical shift appeared at δ 5.46 ppm is due to nitro group substituted in the 4th position of benzyl moiety of nitroimidazole (4-NO_2-C_6H_4-CH_2-*N*). The order of the effect of substituents in the 4th position of benzyl moiety on chemical shift (Fig. 2) is 4-NO_2-C_6H_4-CH_2-*N* (**3f**) > 4-F-C_6H_4-CH_2-*N* (**3d**) > 4-Cl-C_6H_4-CH_2-*N* (**3c**) > 4-Br-C_6H_4-CH_2-*N* (**3b**) > C_6H_5-CH_2-*N* (**3a**) > 4-CH_3-C_6H_4-CH_2-*N* (**3e**).

In comparison of the aromatic protons of 4-substituted phenyl moiety (-C$\mathbf{H}_{\text{phenyl ring}}$), the unsubstituted phenyl moiety of **3a** shows two types of signals. One signal at δ 7.14-7.12 ppm for C_2H and C_6H protons and another one at 7.39-7.37 ppm for C_3H, C_4H and C_5H protons (Fig. 2), whereas 4-bromo substituted phenyl moiety of **3b** shows at δ 7.59-7.57 ppm for C_3H and C_5H protons and chemical shift at δ 7.24-7.22 ppm for C_2H and C_6H protons. The higher chemical shift value clearly indicates the bromo substitution at the 4th position of phenyl moiety. Similar patterns is also observed in case of the 4-chloro substituted phenyl moiety of **3c** at δ 7.46-7.44 ppm for C_3H and C_5H protons and the chemical shift at δ 7.31-7.28 ppm for C_2H and C_6H protons. However, 4-fluoro substituted phenyl moiety of **3d** shows at δ 7.15-7.06 ppm for C_3H and C_5H protons and chemical shift at δ 7.02-7.01 ppm for C_2H and C_6H protons. The lesser chemical shift value clearly indicates to the fluoro substitution at 4th position of phenyl moiety. Again, the similar pattern of ^{1}H NMR for the 4-methyl substituted phenyl moiety of **3e** at δ 7.03-7.01 ppm for C_3H and C_5H protons and chemical shift at δ 7.19-7.17 ppm for C_2H and C_6H protons were observed. In case of compound **3f**, 4-nitro substituted phenyl moiety at δ 8.25-8.23 ppm for C_3H and C_5H protons and less chemical shift at δ 7.51-7.49 ppm for C_2H and C_6H protons were observed too.

For unsubstituted benzyl imidazole moiety (**3a**), the chemical shift of aromatic proton was observed at 7.65 ppm, whereas 4-bromo and 4-chloro substituents containing benzyl imidazole moiety (**3b** and **3c**) have increased their chemical shifts to 8.44 ppm. However, the chemical shift of 4-fluoro and 4-methyl substituents containing benzyl imidazole moiety (**3d** and **3e**) is slightly lowered to 7.64 ppm and 7.61 ppm, respectively and does not affect chemical shift values. The chemical shift at 8.49 ppm is attributed to 4-nitro substituent containing benzyl imidazole moiety (**3f**).

The ^{1}H NMR spectrum of 1-*n*-butyl-2-methyl-4-nitro-1*H*-imidazole (**3g**) showed as singlet peak at δ 8.32 ppm (4-NO_2-isomer) and as singlet peak at δ 2.35 ppm, which are due to aromatic C-H proton (C$\mathbf{H}_{\text{Imidazole ring}}$) and methyl protons (Im-C$\mathbf{H}_3$) of 4-nitroimidazole ring, respectively. The characteristic peak for methylene protons of N-*n*-butyl group (N-CH_2- in **3g**) has appeared at δ 3.98, 3.97, 3.95 ppm as triplet peak (Fig. 3), which has confirmed the formation of *N*-butylation of 2-methyl-4(5)-nitroimidazole (**1a**). Two aliphatic methylene protons are shown as quintet at δ 1.71-1.65 ppm (-CH_2-C$\mathbf{H}_2$-CH_2-N^1<) and as sextet at δ 1.30-1.23 ppm (-CH_3-C$\mathbf{H}_2$-CH_2-). The triplet peak at δ 0.90-0.88 ppm (C$\mathbf{H}_3$-CH_2-), which is assigned to aliphatic methyl protons of *n*-butyl group (Fig. 2).

The ^{1}H NMR spectrum of 1-*sec*-butyl-2-methyl-4-nitro-1*H*-imidazole (**3h**) showed as singlet peak at δ 8.44 ppm (4-NO_2 isomer) and as singlet peak at δ 2.36 and 2.17 ppm, which are due to aromatic C-H proton (C$\mathbf{H}_{\text{Imidazole ring}}$) and methyl protons (Im-C$\mathbf{H}_3$) of 4-nitroimidazole ring, respectively. The characteristic peak for methine proton of N-*sec*-butyl group (N-C**H**- in **3h**) has appeared at δ 4.24-4.18 ppm as sextet peak (Fig. 3), which has confirmed the formation of N-*sec*-butylation of 2-methyl-4(5)-nitroimidazole (**1a**). Two aliphatic methyl protons are shown as doublet at 1.39, 1.38 ppm (C$\mathbf{H}_3$-CH-N^1<) and as triplet at δ 0.94-0.92, 0.76-0.73 ppm (**C**H_3-CH_2-). The quintet peak observed at δ 1.81-1.71 ppm (CH_3-C$\mathbf{H}_2$-CH-N^1<) is assigned to aliphatic methylene proton of *sec*-butyl group (Fig. 3).

The ^{1}H NMR spectrum of 1-*iso*-butyl-2-methyl-4-nitro-1*H*-imidazole (**3i**) showed as singlet peak at δ 8.29 ppm (4-NO_2 isomer), 8.03 ppm (5-NO_2 isomer) and as singlet peak at δ 2.43,

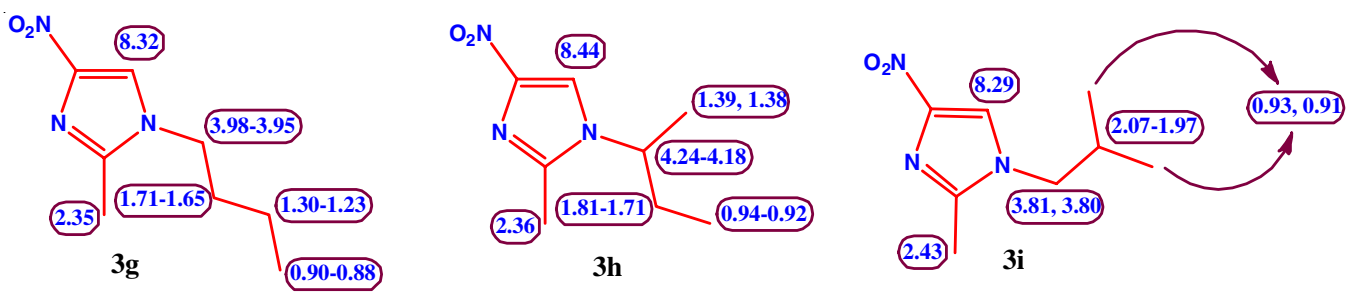

Fig. 3. ^{1}H NMR spectral representation of **3g-i**

2.34 ppm, which are due to aromatic C-H proton ($CH_{Imidazole\ ring}$) and methyl protons (Im-CH_3) of 4-nitroimidazole ring, respectively. The characteristic peak for methylene protons of N-*iso*-butyl group (-CH_2-N in **3i**) has appeared at δ 3.81, 3.80 ppm as doublet peak (Fig. 3), which has confirmed the formation of *n*-butylation of 2-methyl-4(5)-nitroimidazole (**1a**). The aliphatic methine protons are shown as multiplet at 2.07-1.97 ppm (-CH-CH_2N^1<) and the six methyl protons are obtained at 0.93, 0.91 ppm as doublet.

Comparative ^{13}C NMR spectral studies: In comparison of *N*-methylene carbon (4-R-C_6H_4-CH_2-N^1), the unsubstituted benzyl moiety of **3a** showed a peak at 51.05 ppm. The five different substituents (R: Br, Cl, F, CH_3 and NO_2) of benzyl moiety at 4th position are showing slightly lesser chemical shift values (48.93-50.87 ppm) compared to the unsubstituted benzyl moiety of **3a** (51.05 ppm). The chemical shift is observed at 48.99, 48.93, 50.36, 50.87 and 50.36 ppm, which are due to 4-bromo, 4-chloro, 4-fluoro, 4-methyl and 4-nitro benzyl moieties, respectively (Fig. 4). The aromatic carbon (Ph-C_1) of a benzyl moiety of **3a** is shown at 133.91 ppm. The chemical shift value of the 4-halo substituents (R: Br, Cl and F) of the benzyl moiety is shown at 135.04 ppm for 4-bromo derivative (**3b**), 134.62 ppm for 4-chloro derivative (**3c**), 129.32 ppm for 4-fluoro derivative (**3d**), 139.07 ppm for 4-methyl derivative (**3e**) and 143.61 ppm for 4-nitro derivative (**3f**). Among them, 4-nitro substituent (**3f**) has shown a higher chemical shift compared to other substituents (**3a-e**). The aromatic carbon (Ph-C_4) of a benzyl moiety of **3a** has shown at 127.33 ppm (Fig. 4). The chemical shift value of 4-halo substituents (R: Br, Cl and F) of benzyl moiety is shown at 122.49 ppm for 4-bromo derivative (**3b**), 132.76 ppm for 4-chloro derivative (**3c**), 129.77, 129.74 ppm for 4-fluoro derivative (**3d**), 139.07 ppm for 4-methyl derivative (**3e**) and 147.67 ppm for 4-nitro derivative (**3f**). Among them, the 4-nitro substituent (**3f**) shown a higher chemical shift compared to other substituents (**3a-e**). The two equivalent carbons Ph-C_2 and Ph-C_6 and Ph-C_3 and Ph-C_5 of benzyl moiety of **3a** have shown at 128.99 ppm and 129.54 ppm, respectively. These aromatic carbons are observed at 129.66 ppm (Ph-C_2 and Ph-C_6) and 131.82 ppm (Ph-C_3 and Ph-C_5) for 4-bromo substituent present in the benzyl moiety of **3b** (Fig. 4). The chemical shift value of **3b** is slightly higher compared to benzyl moiety of **3a**. These aromatic carbons of 4-chloro substituent present in the benzyl moiety of **3c** are observed at 128.90 ppm (Ph-C_2 and Ph-C_6) and 129.37 ppm (Ph-C_3 and Ph-C_5). It has slightly lesser chemical shift value compared to benzyl moiety of **3a**. The 4-fluoro benzyl moiety has slightly lesser chemical shift value for Ph-C_2 and Ph-C_6 (129.24 ppm) and much lower chemical shift value for Ph-C_3 and Ph-C_5 (116.76, 116.54 ppm) compared to benzyl moiety of **3a** (Fig. 4). The chemical shift of 4-methyl benzyl moiety of **3d** is shown at slightly lowered to 127.41 ppm for Ph-C_2 and Ph-C_6 carbons and slightly increased to 130.80, 130.16 ppm for Ph-C_3 and Ph-C_5 compared to benzyl moiety of **3a**. The similar chemical shift is observed at 128.99 ppm for Ph-C_2 and Ph-C_6 and lowered chemical shift is observed at 124.54 ppm for Ph-C_3 and Ph-C_5 compared to benzyl moiety of **3a**,

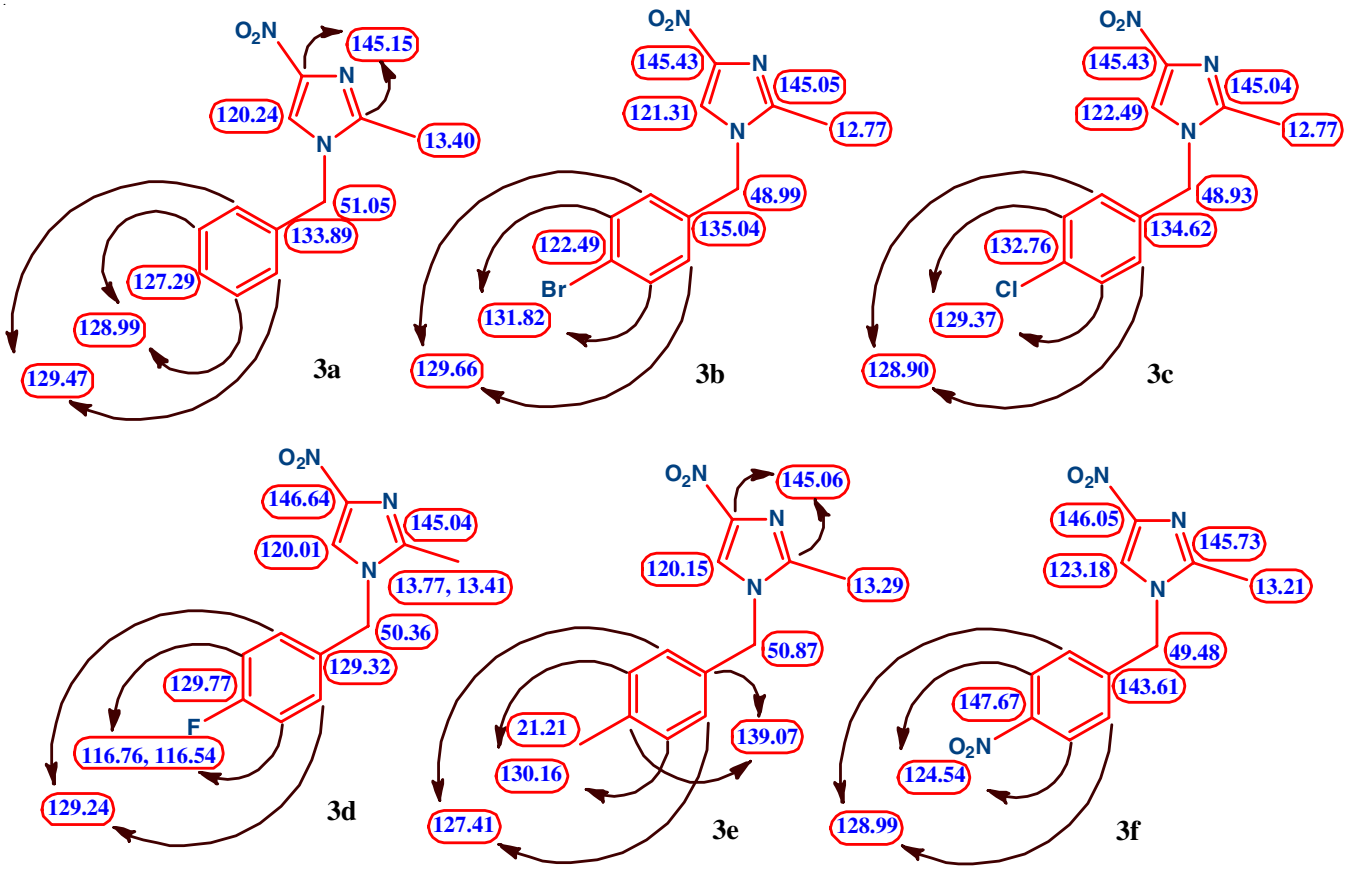

Fig. 4. ^{13}C NMR spectral representation of **3a-f**

which are due to 4-nitro substituent in benzyl moiety of **3f** (Fig. 4).

The chemical shift of the aromatic carbons of unsubstituted benzyl imidazole moiety (**3a**) are shown at 145.15 ppm (Im-$\mathbf{C_2}$ + Im-$\mathbf{C_4}$) and 120.24 ppm (Im-$\mathbf{C_5}$). Except 4-methyl benzyl imidazole (**3e**, 145.06 ppm), all other substituents have enhanced their chemical shift value of Im-$\mathbf{C_4}$ (145.43 ppm for **3b**; 145.43 ppm for **3c**; 146.64 ppm for **3d**; 147.67 ppm for **3f**). Except 4-nitro benzyl imidazole (**3f**, 146.05 ppm), all other substituents have slightly lowered their chemical shift value of Im-$\mathbf{C_2}$ (145.05 ppm for **3b**; 145.04 ppm for **3c**; 145.04 ppm for **3d**; 145.06 ppm for **3e**). The chemical shift of aromatic carbon of Im-$\mathbf{C_5}$ is slightly lowered to120.01 ppm and 120.15 ppm, which are due to 4-fluoro (**3d**) and 4-methyl benzyl moiety (**3e**), respectively. The 4-bromo (**3b**), 4-chloro (**3c**) and 4-nitro benzyl moiety (**3f**) have shown their chemical shift at 121.31 ppm, 122.49 ppm and 123.18 ppm, respectively, which are slightly higher chemical shift value compared to unsubstituted benzyl imidazole moiety (**3a**). The chemical shift value of the methyl protons of imidazole moiety (Im–$\mathbf{CH_3}$) is shown at 13.40 ppm, which is due to unsubstituted benzyl imidazole moiety (**3a**). The 4-methyl benzyl imidazole moiety (**3e**) and 4-nitro benzyl imidazole moiety (**3f**) have shown slightly lowered chemical shift value at 13.29 ppm and 13.21 ppm, respectively. The 4-bromo benzyl imidazole moiety (**3b**) and 4-chloro benzyl imidazole moiety (**3c**) have shown same chemical shift value at 12.77 ppm, which is a low value compared to unsubstituted benzyl imidazole moiety (**3a**). The 4-fluoro benzyl imidazole moiety (**3d**) has shown 13.41 ppm, which is almost the same chemical shift of unsubstituted benzyl imidazole moiety (**3a**).

For the ^{13}C NMR spectrum of **3g**, the aromatic carbons of the 4-nitroimidazole ring were shown at 145.30 ppm (Im-$\mathbf{C_4}$), 144.85 ppm (Im-$\mathbf{C_2}$) and 122.06 ppm (Im-$\mathbf{C_5}$). The methyl carbon of 4-nitroimidazole ring was shown at 12.56 ppm (Im–$\mathbf{CH_3}$). The N-methylene carbon (-$\mathbf{CH_2}$-N^1) showed at 46.15 ppm (Fig. 5). The aliphatic methylene and methyl carbons have appeared at 31.52 ppm (-CH_2-$\mathbf{CH_2}$-CH_2N^1), 19.22, 19.05 ppm (-$\mathbf{CH_2}$-CH_2-CH_2N^1) and 13.47, 13.40 ppm ($\mathbf{CH_3}$-CH_2-CH_2-CH_2N^1). Similarly for **3h**, the aromatic carbons of 4-nitroimidazole ring were shown at 146.02 ppm (Im-$\mathbf{C_4}$), 144.70 ppm (Im-$\mathbf{C_2}$) and 119.03 ppm (Im-$\mathbf{C_5}$). The methyl carbon of the 4-nitroimidazole ring was shown at 12.85 ppm (Im–$\mathbf{CH_3}$). The N-methine carbon (-$\mathbf{CH}$-N^1) showed at 54.29 ppm. The aliphatic methylene and methyl carbons have appeared at 29.29 ppm (CH_3-$\mathbf{CH_2}$-CHN^1), 20.68 ppm ($\mathbf{CH_3}$-CHN^1), 10.22 ppm ($\mathbf{CH_3}$-CH_2-). In **3i**, the aromatic carbons of 4-nitroimidazole ring were shown at 145.31 ppm (Im-$\mathbf{C_4}$), 145.02 ppm (Im-$\mathbf{C_2}$) and 122.35 ppm (Im-$\mathbf{C_5}$). The methyl carbon of 4-nitroimidazole ring was shown at 12.67 ppm (Im–$\mathbf{CH_3}$). The N-methylene carbon (-$\mathbf{CH_2}$-N^1) showed at 53.21 ppm. The aliphatic methine and methyl carbons have appeared at 28.76 ppm (CH_3-$\mathbf{CH}$-CH_2N^1) and 19.23 ppm ($\mathbf{CH_3}$-CH-), respectively.

Anti-inflammatory activity: The N^1-(4-substituted benzyl)-2-methyl-4-nitro-1*H*-imidazoles (**3a-f**) and N^1-butyl-2-methyl-4-nitro-1*H*-imidazoles (**3g-i**) were screened for anti-inflammatory activity in five different concentrations (20, 40, 80, 200 and

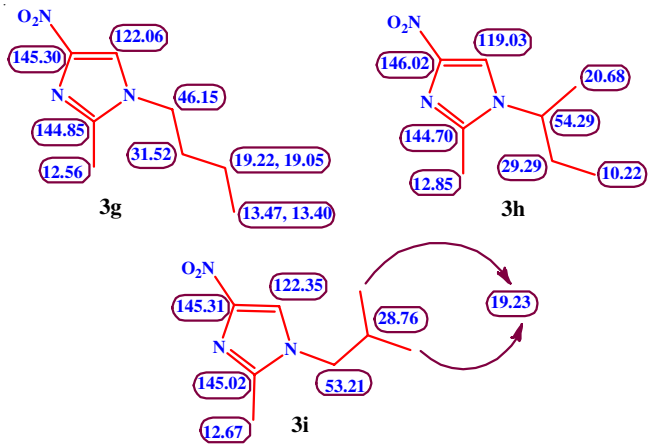

Fig. 5. ^{13}C NMR spectral representation of **3g-i**

400 µg/mL) by using bovine serum albumin (BSA) denaturation method and percentage of inhibition (%) is shown in Fig. 6. Among the tested 4-substituted benzylated imidazoles (**3a-f**), except **3b**, all the imidazols have shown significant anti-inflammatory activity (12.21-17.71, 26.14-30.86, 34.43-38.79, 56.64-60.93 and 70.00-76.00%) compared to standard drug (18.50, 26.21, 39.64, 56.36 and 74.14%). The benzyl moiety containing imidazole (**3a**) shown slightly lesser anti-inflammatory activity (17.71 and 38.79%) at 20 and 80 µg/mL as well as it exposed significant anti-inflammatory activity (30.36, 58.50 and 76.00%) at 40, 200 and 400 µg/mL compared to standard drug (Fig. 6). The 4-bromobenzyl moiety containing imidazole (**3b**) showed less significant anti-inflammatory activity (17.00, 34.71 and 70.00%) in 20, 80 and 400 µg/mL compared to standard drug (18.50, 39.64 and 74.14%). Another two tested concentrations (40 and 200 µg/mL) have shown admirable anti-inflammatory activity (30.86 and 56.64%) compared to standard drug (26.21 and 56.36%). Compound **3b** has shown low anti-inflammatory activity compared to **3a** which has due to benzyl moiety. The 4-chlorobenzyl, 4-methyl and 4-nitrobenzyl moieties containing imidazoles **(3c-f)** have also showed lessanti-inflammatory activity (12.21-15.07%) at 20 µg/mL only compared to standard drug (18.50%). At 40 and 200 µg/mL, the imidazoles **3c-f** have shown high anti-inflammatory activity (26.14-30.07 and 58.14-60.93%)

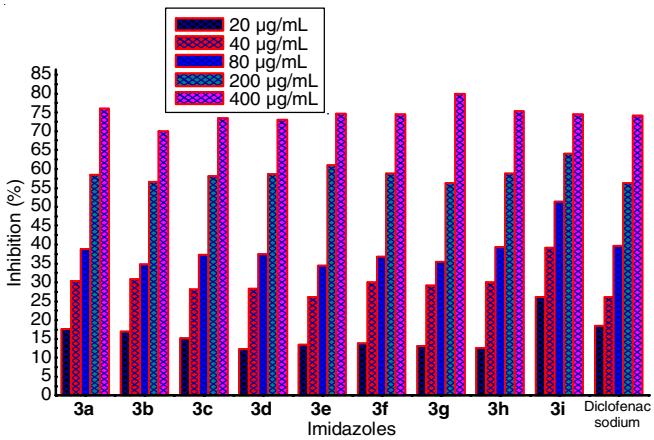

Fig. 6. Anti-inflammatory activity of **3a-i**

compared to standard drug (26.21 and 56.36%). In the tested concentrations 80 and 400 µg/mL, **3c-f** have shown moderate anti-inflammatory activity (34.43-37.43 and 73.07-74.79%) compared to standard drug (39.64 and 74.14%) (Fig. 6).

The *n*-butylated imidazole (**3g**) has shown good anti-inflammatory activity (13.14 and 35.50%) in the tested concentrations of 20 and 80 µg/mL only compared to standard drug (18.50 and 39.64%). In 40, 200 and 400 µg/mL, compound **3g** has shown high anti-inflammatory activity (29.14, 56.21 and 79.93%) compared to standard drug (26.21, 56.36 and 74.14%). Except at 20 and 400 µg/mL, imidazole **3h** has low anti-inflammatory activity compared to **3a** (Fig. 6). The *iso*-butylated imidazole **(3i)** has also shown extraordinary anti-inflammatory activity (26.14, 39.14, 51.29, 64.00 and 74.43%) in all the tested concentrations (20, 40, 80, 200 and 400 µg/mL) compared to standard drug (18.50, 26.21, 39.64, 56.36 and 74.14%).

Antidiabetic activity: The N^1-(4-substituted benzyl)-2-methyl-4-nitro-1*H*-imidazoles (**3a-f**) and N^1-butyl-2-methyl-4-nitro-1*H*-imidazoles (**3g-i**) were also screened for their antidiabetic activity in five different concentrations (20, 40, 80, 200 and 400 µg/mL) by using the α-amylase inhibition assay and the percentage of inhibition (%) and the acarbose was used as a standard drug. The antidiabetic activity of imidazoles **3a-i** and standard drug were shown 16.18, 21.52, 21.34, 13.09, 18.20, 20.78, 16.86, 18.20, 24.45 and 37.36% in 20 µg/mL, respectively (Fig. 7).

Among the tested 4-substituted benzylated imidazole **(3a-f)**, except **3a, 3d** and **3e** (13.09-18.20%), rest of the imidazoles have shown adequate antidiabetic activity (20.78-21.52%) compared to standard drug (37.36%) in 20 µg/mL only (Fig. 7). Except **3d** (25.26%), other imidazoles (**3a-c**, **f**) exhibit satisfactory antidiabetic activity (20.78-21.52%) compared to standard drug (37.36%) in 40 µg/mL only. The *n*-butylated, *sec*-butylated and *iso*-butylated imidazoles (**3g-i**) has shown tolerable antidiabetic activity (16.86-24.45, 36.78-46.16, 47.20-53.17, 56.69-70.69 and 68.23-80.08%) in all the tested concentrations compared to standard drug (37.36, 51.57, 64.22, 81.63 and 85.76%). Among them tested three butylated imidazoles (**3g-i**), the **3i** has shown unforeseen antidiabetic activity compared to **3g** and **3i** in all the tested concentrations.

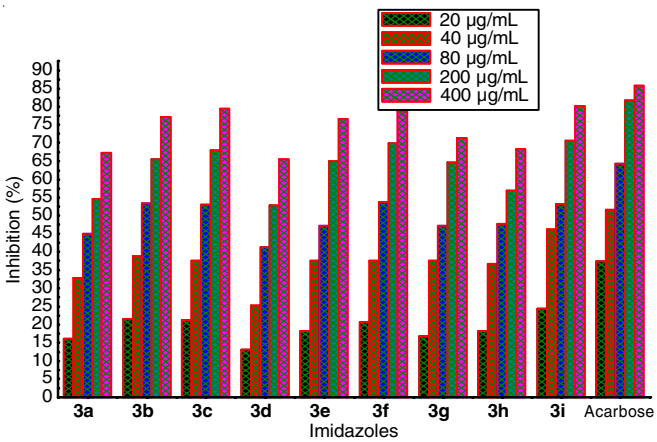

Fig. 7. Antidiabetic activity of **3a-i**

Among imidazoles, **3b, 3c, 3d** and **3i** have shown admirable extraordinary antidiabetic compared to other tested imidazoles (**3a, 3f-h**) as well as standard drug.

Conclusion

In present work, the effects of six different substituents (**3a: H**; **3b: Br**; **3c: Cl**; **3d: F**; **3e: CH_3** and **3f: NO_2**) in the benzyl moieties fourth position and three different butyl substituents (**3g-i: 3g: *n*-C_4H_9-, 3h: *sec*-C_4H_9-** and **3i: *iso*-C_4H_9-**) on the chemical shift values of N^1-(4-substituted benzyl/butyl)-2-methyl-4-nitro-1*H*-imidazoles (**3a-i**) were studied. The 4-nitro benzyl moiety of **3f** showed more effective deviation on its chemical shift values of the *N*-methylene and aromatic protons compared to other substituents (**3a-e**) in the ^{1}H and ^{13}C NMR spectra. The effective deviation on its chemical shift values of compounds **3g-i** with respect to decreases the carbon chain length of the butyl groups. Moreover compounds **3a-i** have also shown the exceptional anti-inflammatory activity when compared to the common drug diclofenac sodium. Similarly, when compared to the common drug acarbose, all the nine imidazoles (**3a-i**) had outstanding antidiabetic 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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