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Synthesis of diethyl 4-substituted-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates as a new series of inhibitors against yeast *a*-glucosidase

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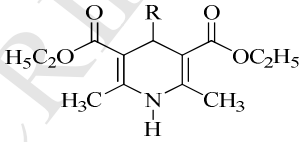
Twenty-five diethyl 4-substituted-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates (**1-25**) were synthesized and their yeast *a*-glucosidase inhibitory activity was evaluated. This lead to the identification of a new series of potent *a*-glucosidase inhibitors. Kinetic studies were also carried out.



1-25

Synthesis of diethyl 4-substituted-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates as a new series of inhibitors against yeast *α*-glucosidase

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Abstract

1,4-Dihydropyridine-3,5-dicarboxylate derivatives (**1**-**25**) were synthesized in high yields *via* Hantzsch reaction and evaluated for their *α*-glucosidase inhibitory activity. Compounds **1**, **2**, **6**, **7**, **8**, **11**, **13**, **14**, **15**, **23**, **24**, and **25** showed a potent inhibitory activity against yeast α-glucosidase with IC_{50} values in the range of 35.0-273.7 μM, when compared with the standard drug acarbose (IC_{50}= 937 ± 1.60 μM). Their structures were characterized by different spectroscopic techniques. The kinetics, selectivity, and toxicity studies on these compounds were also carried out. The kinetic studies on most active compounds **14** and **25** determined their modes of inhibition and dissociation constants K_i. Compound **14** was found to be a non-competitive inhibitor with K_i = 25.0 ± 0.06, while compound **25** was identified as a competitive inhibitor with K_i = 66.0 ± 0.07 μM.

Keywords: 1,4-Dihydropyridine-3,5-dicarboxylates; Hantzsch reaction; *α*-glucosidase inhibition; anti-hyperglycemic activity; type II diabetes

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1. Introduction

The dihydropyridine derivatives exhibit a wide range of biological activities, such as antianxiety, vasodilation, analgesic, neuroprotectant, bronchodilation, anti-inflammatory, anticonvulsant, antidepressant, hypnotic, antitumor, and platelet anti-aggregatory activities [1]. 4-Aryl-1,4-dihydropridines are used in the treatment of hypertension and cardiovascular diseases functioning as calcium channels blockers. These compounds lower the blood pressure by relaxing the smooth muscle wall of heart and arteries and reduce their external resistance [2]. Dialkyl-1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylates are important drugs against hypertension, and angina. Some of them, such as Nitrendipine,® Felodipine,® Isradipine,® Nifedipine,® Lacidipine,® Nimodipine,® Nicardipine,® and Amlodipine® are in clinical use [3].

Hantzsch 1,4-dihydropyridine derivatives serve as reactive moieties in co-enzyme NADH which is a redox reagent in biological system [4]. Based on the biological importance and diverse therapeutic applications of 1,4-dihydropyridines, we evaluated their inhibitory potential against several enzymes. Interestingly they showed a potent activity against yeast α-glucosidase in a biochemical assay.

Diabetes mellitus is a metabolic syndrome, characterized by hyperglycemia [5, 6]. Epidemiological studies by International Diabetes Federation (IDF) showed that more than 375 million people worldwide have diabetes in 2012 [7]. Post-prandial high blood glucose levels, associated with type-2 diabetes mellitus, play a crucial role in the development of atherosclerosis, and cardiovascular disorders [8-9]. α-Glucosidase catalyzes the cleavage of glucose from disaccharides, as only monosaccharides can be absorbed from the intestinal lumen and transported into blood circulation in mammals. Thus, the inhibition of the action of this enzyme in human can be an effective approach to control hyperglycemia in type-2 diabetes [10].

α-Glucosidase inhibitors (AGIs), such as acarbose, miglitol, and voglibose, have sugar like structures, and thus compete with the oligosaccharides for binding to the active site of the enzyme. They effectively decrease the post-prandial glucose levels in type-2 diabetic patients [11]. However, these classic AGIs cause various side effects, such as flatulence, diarrhea, and abdominal discomfort [12]. In addition, all known AGIs have low efficacy with high IC_{50} values against the enzyme. Due to the crucial role of this enzyme in hyperglycemia, and low tolerability of the existing AGIs, there is a need to discover safe and efficient inhibitors for the effective control of hyperglycemia in diabetes.

We describe here the synthesis and characterization of diethyl 4-substituted-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates (**1-25**). These compounds were first time evaluated for their *α*-glucosidase inhibitory activity through a biochemical mechanism-based assay. Their kinetics, selectivity, and toxicity were also evaluated. The kinetic studies on the most active compounds **14** and **25** determined their modes of inhibition and dissociation constants K_i. Compound **14** a non-competitive inhibitor showed a K_i = 25.0 ± 0.06, however, compound **25** was identified as a competitive inhibitor with a K_i = 66.0 ± 0.07 μM. The selectivity was checked against phosphodiestrase-I, carbonic anhydrase-II, and *β*-glucuronidase, and no activity was observed. The cytotoxicity was checked against 3T3 cell line and no toxicity was observed.

2. Results and discussion

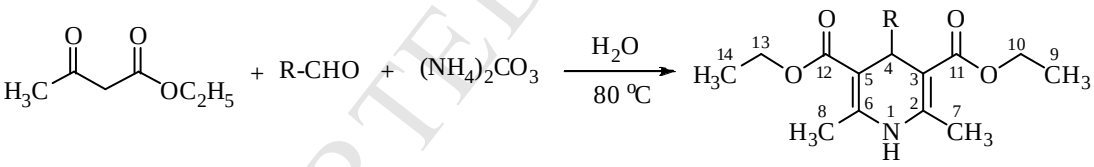
2.1. Chemistry

Multi-component reactions (MCR) form an important sub-class of tandem reactions. They are suitable for green syntheses as three or more components react efficiently to form a product in good yield [13]. Due to their intrinsic atom economy, selectivity, simplicity, time and energy

efficiency, as well as eco-friendliness, MCRs are gaining importance in research and development [14].

Hantzsch MCR reaction has attracted much scientific attention in the synthesis of 1,4-dihydropyridines (1,4-DHPs) [15]. The mechanism involves a Knoevenagel condensation of the ethylacetoacetate with the aldehyde to form an *α,β*-unsaturated carbonyl compound, and condensation of ammonia (produced *in situ* from $(NH_4)_2CO_3$) with another equivalent of the ethylacetoacetate to yield an enaminoester. The Michael addition of the enaminoester to *α,β*-unsaturated carbonyl compound, and a finally intramolecular condensation results in the formation of desired diethyl 4-aryl/alkyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates.

Compounds **1-25** were synthesized by multi-component condensation reaction of ethyl acetoacetate with different aliphatic, and aromatic aldehydes in water using ammonium carbonate as a solid ammonia source (Scheme-1) [16].



1-25

Scheme-1: Synthesis of 1,4-dihydropyridine-3,5-dicarboxylate derivatives **1-25**

Aromatic aldehydes, containing either electron withdrawing or electron donating substituents, aliphatic, and heteroaryl aldehydes, were reacted efficiently with ethyl acetoacetate in the presence of ammonium carbonate. Desired products were obtained in generally good yields. The structures of synthetic compounds were confirmed by ^{1}H-NMR, EI and HREI-MS spectroscopy and CHN analyses. Compounds **1-25** have been previously reported [17-30].

2.2. Biological activity

Compounds **1-25** were evaluated for their inhibitory potential against the α-glucosidase enzyme (EC 3.2.1.20, *Saccharomyces cerevisiae*) (Table-1) and showed significant activity with IC_{50} values in the range 35.0 ± 1.01 to 273.7 ± 1.04 μM (Table-1). The 4-substituted-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates **1**, **2**, **6**, **7**, **8**, **11**, **13**, **14**, **15**, **23**, **24**, and **25** were found to be active, while rest of the compounds showed less than 50% inhibition and were not screened for their IC_{50} values. Compounds **14** and **25** were about 26- and 18-folds more active with IC_{50} values of 35.0 ± 0.17 and 51 ± 0.98 μM, respectively, as compared to the standard drug acarbose (IC_{50} = 937 ± 1.60 μM).

Insert Table-1 here

Insert Figure-1 here

Limited SAR suggests that the activity mainly depends upon the presence of R (aliphatic or aromatic) group and in case of aromatic, nature and position of substituents on it (**Figure-1**). Compounds **14** (IC_{50} = 35.0 ± 0.17 μM) and **25** (IC_{50} = 51 ± 0.98 μM) containing nitro group at phenyl ring showed a potent activity. The enhanced activity of compound **14** may be due to presence of an electron-withdrawing NO_2 group at *meta* position which may be responsible for its binding with enzyme. In comparision relatively decreased activity of compound **25** may be due to change in the position of NO_2 from *meta* to *para*. Compounds **11** (IC_{50} = 60.2 ± 1.01 μM) having small fluorine group at *ortho* position and **1** (IC_{50} = 72.4 ± 1.03 μM) having a relatively large chlorine residues at *para* position are the determing factor for difference in activities. The presence of bulky carboxyl moiety at *ortho* position of phenyl ring made the derivative **18** inactive against the enzyme. Compounds containing *para* substituted phenyl moiety were also

found to be active. In addition, compound **5** containing unsubstituted phenyl ring, was found to be inactive.

When *meta* position is substituted with an electron-donating hydroxyl group, the resulting compound **17** showed no activity, however, derivative **23** have both *meta* and *para* hydroxyl substitution showed a weak inhibition (IC_{50} = 273.7 ± 1.04 μM) as compare to other active compounds. Same pattern was observed in compounds **8**, **13**, and **16.** Compounds **13** and **8** with a methoxy/ethoxy group at *para* position, showed good activities (IC_{50} = 65.1 ± 0.92 and IC_{50} = 124.3 ± 0.97 μM, respectively), but when another methoxy was introduced at *meta* position, the resulting compound **16** became inactive.

When R group was changed to aliphatic moieties as in analogues **4**, **19**-**22**, the resulting compounds displayed no activity against the enzyme. However, in case of compound **24** where ethyl benzene moiety was present as R made the derivative potent inhibitor of the enzyme (IC_{50} = 112 ± 0.64 μM). This difference in activitity suggested that remote phenyl ring may be helful for its binding with active site of the enzyme.

Heterocyclic residues containing analogues **3**, **9**, **10** and **12** were found to be inactive, except sulphur containing compounds **2** (IC_{50} = 74.3 ± 0.76 μM) and **6** (IC_{50} = 97.4 ± 0.32 μM) showed good activity.

From aforementioned discussion it can easily be extracted that the yeast α-glucosidase inhibitory activity of diethyl 4-substituted-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylates (**1**-**25**) is depending upon the nature of R (aliphatic or aromatic) group and in case of aromatic, position and effect of substituents on it.

2.3. Kinetic studies

To study the mechanism of inhibition, the kinetic studies of the most active compounds **14** and **25** were performed. In the kinetic studies, different concentrations of test compounds and substrates were used. The result indicated that compound **14** is a non-competitive inhibitor with K_i value 25 ± 0.06 μM, while compound **25** is a competitive inhibitor with K_i value 68 ± 0.07 μM.

The type of inhibition was determined by Lineweaver-Burk plots, the reciprocal of the rate of the reaction were plotted against the reciprocal of substrate concentrations to monitor the effect of inhibitor on both, K_m and V_{max}. Figure-2A showed that V_{max} of enzyme decreased without affecting the K_m of enzyme which indicated that compound **14** is a non-competitive inhibitor of α-glucosidase.

The secondary re-plots of Lineweaver-Burk plots were plotted to determine the *Ki* values (Figure-2B). The *Ki* value was calculated by plotting the slope of each line in the Lineweaver-Burk plots against the different concentrations of compound **14**. The *Ki* value was confirmed from Dixon plot by plotting the reciprocal of the rate of reaction against different concentrations of compound **14** (Figure-2C).

Insert Figure-2 here

The kinetic studies showed that the compound **25** is a competitive inhibitor of α-glucosidase (Figure-3). Figure-3A showed that the V_{max} of enzyme was not affected with varying concentrations of compound **25**, while the K_m increased. This indicated a pure competitive-type of inhibition.

Insert Figure-3 here

2.4. Selectivity

In order to asses the selectivity of the compounds for α-glucosidase, the most potent compounds **2**,**13**,**14**, and **25** were evaluated for their inhibitory activity against the phosphodiestrase-I [31], carbonic anhydrase-II [32], and β-glucuronidase enzymes [33], and no activity was observed (Table-2).

Insert Table-2 here

2.5. Cytotoxicity

The cytotoxicity of compounds **1**, **2**, **6-8**, **11**, **13**, **14**, and **23-25** was evaluated against 3T3 cell line [34]. Most of these compounds exhibited a non-toxic behavior, except compounds **1**, **23**, and **24** which showed low cytotoxicity with IC_{50} values of 27.857 ± 0.25, 28.634 ± 0.24, and 26.726 ± 0.52, respectively (Table-3).

Insert Table-3 here

3. Conclusion

Compounds **1**, **2**, **6-8**, **11**, **13-15**, and **23-25** showed a potent inhibitory activity against yeast α-glucosidase with IC_{50} values in the range of 35 ± 0.17 to 273.7 ± 1.04 μM, when compared with the standard drug acarbose (IC_{50} = 937 ± 1.60 μM). Limited SAR studies indicated that *meta* position in aromatic aldehydes is important for activity. Introduction of electron withdrawing groups at *meta* position enhanced the activity, while introduction of electron donating groups at *meta* position, resulted in either decrease or loss of activity. Compounds containing nitro group, halogens or sulphur expressed a strong inhibitory potential against the yeast α-glucosidase enzyme, therefore future development or structural modifications of these

moieties are requird to explore inhibitory mechanism. However, these molecules needs to be further evaluated through animal model, i.e. *in vivo* studies to establish their therapeutic potential to overcome the pathologies in diabetes.

4. Materials and methods

NMR experiments were performed on Avance Bruker AM 300, 400, 500, and 600 MHz instruments, Rheinstetten, Germany. CHN analyses were carried on a Carlo Erba Strumentazione-Mod-1106, Italy, apparatus, and electron impact mass spectra (EI-MS) on a Finnigan MAT-311A, instrument, Bremen, Germany. Thin-layer chromatography (TLC) was performed on pre-coated silica gel glass plates (Kieselgel 60, 254, E. Merck, Darmstadt, Germany). Chromatograms were visualized by either UV at 254 or 365 nm. Melting points (uncorrected) were recorded on a Büchi Labortechnik AG, Switzerland, melting point apparatus model B-540.

*4.1. General procedure for the synthesis of 1,4-dihydropyridine-3,5-dicarboxylate derivatives **1**-**25***

A mixture of aldehyde (1 mmol), ethyl acetoacetate (2 mmol) and ammonium carbonate (2 mmol) was stirred in water at 80 °C for 3 to 5 h. The progress of reaction was monitored through the TLC analysis. After completion of the reaction as indicated by TLC, the mixture was diluted with cold H_2O, precipitated product was filtered, washed with distilled water, and crystallized from absolute ethanol.

*4.1.1. Diethyl 4-(4-chlorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**1**)*

Yield: 85%; M.p.: 145-147 °C (Lit. M.p.[24] 147-150 °C); $R_{f:}$ 0.67 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.84 (s, 1H, NH), 7.24 (d, 2H, $J_{2',3'} = J_{6',5'} = 8.4$ Hz, H-2′/H-6′), 7.13 (d, 2H, $J_{3',2'} = J_{5',6'} = 8.4$ Hz, 2H, H-3′/H-5′), 4.82 (s, 1H, H-4), 3.98 (m, 4H, $2CH_2$),

2.24 (s, 6H, $2CH_3$), 1.11 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 166.7 (C-11 and C-12, C=O), 147.0 (C-1′, C-Ar), 145.5 (C-2 and C-6, C=C), 130.3 (C-4′, C-Ar), 129.1 (C-2′ and C-6′, C-Ar), 127.7 (C-3′ and C-5′, C-Ar), 101.4 (C-3 and C-5, C=C), 59.0 (C-10 and C-13, OCH_2), 38.4 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 365 (M^+ +2, 8.3), 364 (M^++1, 9.1), 363 (M^+, 23.6), 252 (100), 224 (66), 196 (71), 150 (21); HREI-MS: *m/z* Calcd for $C_{19}H_{22}ClNO_4$: 363.1237; Found: 363.1202; Anal. Calcd for $C_{19}H_{22}ClNO_4$ (363.84): C, 62.72; H, 6.09; Cl, 9.74; N, 3.85; O, 17.59; Found:C, 62.77; H, 6.04; N, 3.82.

*4.1.2. Diethyl 2,6-dimethyl-4-(2-thienyl)-1,4-dihydropyridine-3,5-dicarboxylate (**2**)*

Yield: 87%; M.p.: 154-156 °C (Lit. M.p.[20] 153-155 °C); R_f: 0.59 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.99 (s, 1H, NH), 7.17 (dd, 1H, $J_{5',3'}$ = 0.9 Hz, $J_{5',4'}$ = 4.8 Hz, H-5′), 6.81 (dd, 1H, $J_{4',3'}$ = 3.3 Hz, $J_{4',5'}$ = 4.8 Hz, H-4′), 6.64 (d, 1H, $J_{3',4'}$ = 3.3 Hz, H-3′), 5.16 (s, 1H, H-4), 4.05 (m, 4H, $2CH_2$), 2.25 (s, 6H, $2CH_3$), 1.11 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 166.6 (C-11 and C-12, C=O), 151.8 (C-2′, C-Ar), 145.8 (C-2 and C-6, C=C), 126.3 (C-4′, C-Ar), 123.3 (C-3′, C-Ar), 122.4 (C-5′, C-Ar), 101.3 (C-3 and C-5, C=C), 59.1 (C-10 and C-13, OCH_2), 33.7 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.2 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 337 (M^+ +2, 1.6), 335 (M^+, 20), 137 (100), 109 (52), 65 (12); HREI-MS: *m/z* Calcd for $C_{17}H_{21}NO_4S$: 335.1191; Found: 335.1199; Anal. Calcd for $C_{17}H_{21}NO_4S$ (335.42): C, 60.87; H, 6.31; N, 4.18; O, 19.08; S; 9.56; Found:C, 60.83; H, 6.28; N, 4.21.

*4.1.3. Diethyl 2,6-dimethyl-4-(3-pyridinyl)-1,4-dihydropyridine-3,5-dicarboxylate (**3**)*

Yield: 80%; M.p.: 191-192 °C (Lit. M.p.[16] 190-192 °C); R_f: 0.21 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.91 (s, 1H, NH), 8.35 (br. d, 1H, $J_{2',4'} = J_{2',6'} = 1.5$ Hz, H-2′), 8.29 (br. d, 1H, $J_{4',5'} = 3.9$ Hz, H-4′), 7.48 (br. d, 1H, $J_{6',5'} = 7.8$ Hz, H-6′), 7.23 (dd, 1H, $J_{5',4'} = 4.8$ Hz, $J_{5',6'} = 7.8$ Hz, H-5′), 4.82 (s, 1H, H-4), 3.97 (m, 4H, $2CH_2$), 2.25 (s, 6H, $2CH_3$), 1.10 (t, 6H, $J = 7.2$ Hz, $2CH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 166.5 (C-11 and C-12, C=O), 148.7 (C-6′, C-Ar), 147.1 (C-2′, C-Ar), 146.0 (C-2 and C-6, C=C), 143.2 (C-3′, C-Ar), 134.8 (C-4′, C-Ar), 123.4 (C-5′, C-Ar), 101.0 (C-3 and C-5, C=C), 59.0 (C-10 and C-13, OCH_2), 36.9 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 330 (M^+, 6.3), 252 (100), 224 (28), 196 (50), 150 (7.6); HREI-MS: *m/z* Calcd for $C_{18}H_{22}N_2O_4$: 330.1580; Found: 330.1571; Anal. Calcd for $C_{18}H_{22}N_2O_4$ (330.37): C, 65.44; H, 6.71; N, 8.48; O, 19.37; Found: C, 65.39; H, 6.68; N, 8.51.

*4.1.4. Diethyl 4-ethyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**4**)*

Yield: 78%; M.p.: 111-112 °C (Lit. M.p.[28] 112 °C); R_f: 0.67 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.58 (s, 1H, NH), 4.04 (m, 4H, $2CH_2$), 3.73 (t, 1H, $J = 5.4$ Hz, H-4), 2.18 (s, 6H, $2CH_3$), 1.18 (t, 6H, $J = 7.2$ Hz, $2CH_3$), 1.21 (m, 2H, aliphatic-CH_2), (t, 3H, $J = 7.5$ Hz, aliphatic- CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 167.2 (C-11 and C-12, C=O), 146.1 (C-2 and C-6, C=C), 100.2 (C-3 and C-5, C=C), 58.7 (C-10 and C-13, OCH_2), 33.3 (C-4, CH), 29.0 (aliphatic-$\underline{CH_2}CH_3$), 18.1 (C-7 and C-8, CH_3), 14.3 (C-9 and C-14, $OCH_2\underline{CH_3}$), 8.9 (aliphatic-$CH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 281 (M^+), 252 (100), 236 (42), 224 (72); HREI-

MS: *m/z* Calcd for $C_{15}H_{23}NO_4$: 281.1627; Found: 281.1633; Anal. Calcd for $C_{15}H_{23}NO_4$ (281.34): C, 64.03; H, 8.24; N, 4.98; O, 22.75; Found:C, 64.06; H, 8.19; N, 5.01.

*4.1.5. Diethyl 2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (**5**)*

Yield: 85%; M.p.: 159-160 °C (Lit. M.p.[24] 158-160); R_f: 0.59 (*n*-hexane/ethylacetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.78 (s, 1H, NH), 7.14 (m, 5H, aromatic), 4.84 (s, 1H, H-4), 3.99 (m, 4H, $2CH_2$), 2.24 (s, 6H, $2CH_3$), 1.11 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 166.9 (C-11 and C-12, C=O), 148.1 (C-1′, C-Ar), 145.2 (C-2 and C-6, C=C), 127.7 (C-3′ and C-5′, C-Ar), 127.2 (C-2′ and C-6′, C-Ar), 125.8 (C-4′, C-Ar), 101.8 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 38.8 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 330 (M^++1, 3.8), 329 (M^+, 15.2), 252 (100), 224 (43), 196 (55), 150 (9.2); HREI-MS: *m/z* Calcd for $C_{19}H_{23}NO_4$: 329.1627; Found: 329.1639; Anal. Calcd for $C_{19}H_{23}NO_4$ (329.39): C, 69.28; H, 7.04; N, 4.25; O, 19.43; Found: C, 69.30; H, 7.01; N, 4.22.

*4.1.6. Diethyl 2,6-dimethyl-4-[4-(methylsulfanyl)phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (**6**)*

Yield: 92%; M.p.: 140-142 °C (Lit. M.p.[24] 138-141 °C); R_f: 0.69 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.78 (s, 1H, NH), 7.10 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.7 Hz, H-2′/H-6′), 7.04 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.7 Hz, 2H, H-3′/H-5′), 4.79 (s, 1H, H-4), 3.95 (m, 4H, $2CH_2$), 2.39 (s, 3H, $-SCH_3$), 2.23 (s, 6H, $2CH_3$), 1.12 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 166.8 (C-11 and C-12, C=O), 145.2 (C-2 and C-6, C=C), 145.0 (C-4′, C-Ar), 135.0 (C-1′, C-Ar), 127.9 (C-2′ and C-6′, C-Ar), 125.7 (C-3′ and C-5′, C-Ar), 101.7 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 38.3 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.8 ($-SCH_3$),

14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 376 (M^++1, 7.4), 375 (M^+, 35), 373 (62), 252 (100), 224 (32), 196 (43), 150 (10), 83 (27); HREI-MS: *m/z* Calcd for $C_{20}H_{25}NO_4S$: 375.1504; Found: 375.1509; Anal. Calcd for $C_{20}H_{25}NO_4S$ 375.48): C, 63.97; H, 6.71; N, 3.73; O, 17.04; S, 8.54; Found:C, 63.92; H, 6.75; N, 3.70.

*4.1.7. Diethyl 2,6-dimethyl-4-(4-methylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**7**)*

Yield: 83%; M.p.: 132-133 °C (Lit. M.p.[29] 130-132 °C); R_f: 0.66 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.73 (s, 1H, NH), 7.01 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.4 Hz, H-2′/H-6′), 6.97 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.4 Hz, H-3′/H-5′), 4.79 (s, 1H, H-4), 3.95 (m, 4H, $2CH_2$), 2.22 (s, 6H, $2CH_3$), 2.19 (s, 3H, aromatic-CH_3), 1.11 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 166.9 (C-11 and C-12, C=O), 145.2 (C-1′, C-Ar), 145.0 (C-2 and C-6, C=C), 134.7 (C-4′, C-Ar), 128.3 (C-2′ and C-6′, C-Ar), 127.1 (C-3′ and C-5′, C-Ar), 101.9 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 38.3 (C-4, CH), 20.5 (Ar-CH_3), 18.1 (C-7 and C-8, CH_3), 14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 344 (M^++1, 2), 343 (M^+, 9), 252 (100), 224 (56), 196 (62), 150 (20), 91 (12); HREI-MS: *m/z* Calcd for $C_{20}H_{25}NO_4$: 343.1784; Found: 343.1785; Anal. Calcd for $C_{20}H_{25}NO_4$ (343.42): C, 69.95; H, 7.34; N, 4.08; O, 18.64; Found: C, 69.98; H, 7.32; N, 4.03.

*4.1.8. Diethyl 4-(4-ethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**8**)*

Yield: 77%; M.p.: 104-106 °C (Lit. M.p.[25] 104-106 °C); R_f: 0.57 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.73 (s, 1H, NH), 7.01 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.7 Hz, H-2′/H-6′), 6.72 (d, 2H, $J_{3',2'} = J_{5',6'}$ = 8.7 Hz, H-3′/H-5′), 4.77 (s, 1H, H-4), 4.01 (m, 6H, $3CH_2$), 2.22 (s, 6H, $2CH_3$), 1.26 (t, 3H, J = 6.0, CH_3), 1.11 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (100

MHz, DMSO-d_6): δ 166.9 (C-11 and C-12, C=O), 156.6 (C-4′, C-Ar), 144.8 (C-2 and C-6, C=C), 140.3 (C-1′, C-Ar), 128.2 (C-2′ and C-6′, C-Ar), 113.6 (C-3′ and C-5′, C-Ar), 102.0 (C-3 and C-5, C=C), 62.7 (Ar-OCH_2), 58.8 (C-10 and C-13, OCH_2), 37.8 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.6 (Ar-$OCH_2\underline{CH_3}$), 14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 374 (M^++1, 7), 373 (M^+, 27), 344 (60), 300 (64), 252 (100), 224 (62), 196 (67), 150 (22) ; HREI-MS: *m/z* Calcd for $C_{21}H_{27}NO_5$: 373.1889; Found: 373.1882; Anal. Calcd for $C_{21}H_{27}NO_5$ (373.44): C, 67.54; H, 7.29; N, 3.75; O, 21.42; Found: C, 67.51; H, 7.32; N, 3.72.

*4.1.9. Diethyl 2,6-dimethyl-4-(1H-pyrrol-2-yl)-1,4-dihydropyridine-3,5-dicarboxylate (**9**)*

Yield: 88%; M.p.: 214-216 °C; R_f: 0.50 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 9.73 (s, 1H, NH), 8.74 (s, 1H, NH), 6.42 (d, 1H, $J_{3',4'}$ = 1.8 Hz, H-3′), 5.76 (d, 1H, $J_{4',3'}$ = 2.4 Hz, H-4′), 5.52 (br. s, 1H, H-5′), 4.87 (s, 1H, H-4), 4.05 (m, 4H, $2CH_2$), 2.21 (s, 6H, $2CH_3$), 1.15 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 167.1 (C-11 and C-12, C=O), 145.2 (C-2 and C-6, C=C), 137.1 (C-2′, C-Ar), 115.9 (C-5′, C-Ar), 106.7 (C-4′, C-Ar), 103.6 (C-3′, C-Ar), 100.4 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 31.8 (C-4, CH), 18.3 (C-7 and C-8, CH_3), 14.2 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS:*m/z* (rel. abund. %), 319 (M^++1, 6), 318 (M^+, 27), 252 (13), 245 (22), 206 (100), 195 (20), 178 (34), 150 (16); HREI-MS: *m/z* Calcd for $C_{17}H_{22}N_2O_4$: 318.1580; Found: 318.1566; Anal. Calcd for $C_{17}H_{22}N_2O_4$ (318.37): C, 64.13; H, 6.97; N, 8.80; O, 20.10; Found:C, 64.10; H, 6.99; N, 8.77.

*4.1.10. Diethyl 4-(2-furyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**10**)*

Yield: 92%; M.p.: 160-162 °C (Lit. M.p.[24] 159-161 °C); R_f: 0.57 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.89 (s, 1H, NH), 7.37 (s, 1H, H-3′), 6.23 (dd, 1H, $J_{4',3'}$

= 2.1 Hz, $J_{4',5'}$ = 3.0 Hz, H-4′), 5.81 (d, 1H, $J_{5',4'}$ = 3.0 Hz, H-5′), 5.03 (s, 1H, H-4), 4.04 (m, 4H, $2CH_2$), 2.23 (s, 6H, $2CH_3$), 1.15 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 166.7 (C-11 and C-12, C=O), 158.7 (C-2′, C-Ar), 146.3 (C-2 and C-6, C=C), 141.0 (C-5′, C-Ar), 110.1 (C-4′, C-Ar), 103.9 (C-3′, C-Ar), 98.4 (C-3 and C-5, C=C), 59.0 (C-10 and C-13, OCH_2), 32.7 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.2 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 321 (M^+ +2, 6), 319 (M^++1, 37), 319 (M^+, 73), 290 (72), 274 (69), 262 (68), 252 (68), 246 (100), 218 (70), 196 (66), 150 (50); HREI-MS: *m/z* Calcd for $C_{17}H_{21}NO_5$: 319.1420; Found: 319.1394; Anal. Calcd for $C_{17}H_{21}NO_5$(319.35): C, 63.94; H, 6.63; N, 4.39; O, 25.05; Found:C, 63.91; H, 6.61; N, 4.40.

*4.1.11. Diethyl 4-(2-fluorophenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**11**)*

Yield: 75%; M.p.: 147-149 °C (Lit. M.p.[20] 148-152 °C); R_f: 0.60 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.79 (s, 1H, NH), 7.20 (td, 1H, $J_{5',3'}$ = 1.8 Hz, $J_{5',4'}$ = $J_{5',6'}$ = 7.5 Hz, H-5′), 7.11 (m, 1H, H-4′), 7.03 (dd, 1H, $J_{3',5'}$ = 1.8 Hz, $J_{3',4'}$ = 7.2 Hz, H-3′), 6.96 (br. d, 1H, $J_{6',5'}$ = 8.4 Hz, H-6′), 3.94 (m, 4H, $2CH_2$), 2.22 (s, 6H, $2CH_3$), 1.07 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (150 MHz, DMSO-d_6): δ 166.7 (C-11 and C-12, C=O), 159.4 (d, J = 247.5 Hz, C-2′, C-Ar), 145.7 (C-2 and C-6, C=C), 135.5 (d, J = 15.0 Hz, C-1′, C-Ar), 130.6 (d, J = 3.0 Hz, C-6′, C-Ar), 127.7 (d, J = 7.5 Hz, C-4′, C-Ar), 124.0 (C-5′, C-Ar), 114.6 (d, C-3′, J = 24.0 Hz, C-Ar), 101.0 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 33.1 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 13.9 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 347 (M^+, 6), 274 (22), 252 (100), 224 (36), 196 (50), 150 (13); HREI-MS: *m/z* Calcd for $C_{19}H_{22}NO_4F$: 347.1533 Found: 347.1513; Anal. Calcd for $C_{19}H_{22}NO_4F$ (347.38): C, 65.69; H, 6.38; F, 5.47; N, 4.03; O, 18.42; Found:C, 65.72; H, 6.35; N, 4.01.

*4.1.12. Diethyl 2,6-dimethyl-4-(5-methyl-2-furyl)-1,4-dihydropyridine-3,5-dicarboxylate (**12**)*

Yield: 93%; M.p.: 132-134 °C; R_f: 0.58 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.83 (s, 1H, NH), 5.81 (d, 1H, $J_{3',4'}$ = 2.7 Hz, H-3′), 5.64 (d, 1H, $J_{4',3'}$ = 2.7 Hz, H-4′), 4.95 (s, 1H, H-4), 4.06 (m, 4H, $2CH_2$), 2.22 (s, 6H, $2CH_3$), 2.11 (s, 3H, CH_3), 1.16 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 166.7 (C-11 and C-12, C=O), 157.2 (C-2′, C-Ar), 149.3 (C-5′, C-Ar), 146.1 (C-2 and C-6, C=C), 106.0 (C-3′, C-Ar), 104.6 (C-4′, C-Ar), 98.6 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 32.7 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.2 (C-9 and C-14, $OCH_2\underline{CH_3}$), 13.4 (Ar-CH_3); EI-MS: *m/z* (rel. abund. %), 335 (M^+ +2, 2), 334 (M^++1, 10), 333 (M^+, 54), 304 (39), 260 (100), 252 (11), 232 (14), 214 (14), 196 (7) ; HREI-MS: *m/z* Calcd for $C_{18}H_{23}NO_5$: 333.1576; Found: 333.1581; Anal. Calcd for $C_{18}H_{23}NO_5$ (333.38): C, 64.85; H, 6.95; N, 4.20; O, 24.00; Found:C, 64.80; H, 6.92; N, 4.25.

*4.1.13. Diethyl 4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**13**)*

Yield: 85%; M.p. 160-161 °C (Lit. M.p.[24] 158-161 °C); R_f: 0.56 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.73 (s, 1H, NH), 7.03 (d, 2H, $J_{2',3'} = J_{6',5'}$ = 8.4 Hz, H-2′/H-6′), 6.74 (d, $J_{3',2'} = J_{5',6'}$ = 8.4 Hz, 2H, H-3′/H-5′), 4.78 (s, 1H, H-4), 3.97 (m, 4H, $2CH_2$), 3.66 (s, 3H, -OCH_3), 2.23 (s, 6H, $2CH_3$), 1.12 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 166.9 (C-11 and C-12, C=O), 157.4 (C-4′, C-Ar), 144.9 (C-2 and C-6, C=C), 140.4 (C-1′, C-Ar), 128.2 (C-2′ and C-6′, C-Ar), 113.1 (C-3′ and C-5′, C-Ar), 102.1 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 54.8 (Ar-OCH_3), 37.9 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 361 (M^++2, 1.3), 360 (M^++1, 6.5), 359 (M^+, 35), 330 (46), 286 (82), 252 (100), 224 (45), 196 (51), 150 (13), 43 (9); HREI-MS: *m/z* Calcd for

$C_{20}H_{25}NO_5$: 359.1733; Found: 359.1755; Anal. Calcd for $C_{20}H_{25}NO_5$ (359.42): C, 66.83; H, 7.01; N, 3.90; O, 22.26; Found: C, 66.80; H, 6.98; N, 3.94.

*4.1.14. Diethyl 2,6-dimethyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**14**)*

Yield: 90%; M.p.: 163-164 °C (Lit. M.p.[24] 162-165 °C); R_f: 0.53 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.99 (s, 1H, NH), 7.98 (dd, 1H, $J_{2',4'}$ = 0.9 Hz, $J_{2',6'}$ = 2.1 Hz, H-2′), 7.55 (m, 3H, H-4′/5′/6′), 4.94 (s, 1H, H-4), 4.94 (s, 1H, H-4), 3.97 (m, 4H, 2CH_2), 2.27 (s, 6H, 2CH_3), 1.10 (t, 6H, J = 7.2 Hz, 2CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 166.4 (C-11 and C-12, C=O), 150.2 (C-3′, C-Ar), 147.3 (C-1′, C-Ar), 146.3 (C-2 and C-6, C=C), 134.1 (C-2′, C-Ar), 129.5 (C-4′, C-Ar), 121.8 (C-6′, C-Ar), 121.0 (C-5′, C-Ar), 101.0 (C-3 and C-5, C=C), 59.1 (C-10 and C-13, OCH_2), 39.2 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.0 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 374 (M^+, 4), 252 (100), 224 (21), 196 (33), 176 (14), 150 (10), 43 (25); HREI-MS: *m/z* Calcd for $C_{19}H_{22}N_2O_6$: 374.1478; Found: 374.1483; Anal. Calcd for $C_{19}H_{22}N_2O_6$ (374.15): C, 60.95; H, 5.92; N, 7.48; O, 25.64; Found: C, 60.92; H, 5.96; N, 7.43.

*4.1.15. Diethyl 4-butyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**15**)*

Yield: 85%; M.p.: 94-95 °C (Lit. M.p.[24] 92-94 °C); R_f: 0.75 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO- d_6): δ 8.61 (s, 1H, NH), 4.02 (m, 4H, 2CH_2), 3.75 (t, 1H, J = 5.7, H-4), 2.18 (s, 6H, 2CH_3), 1.18 (t, 6H, J = 7.2 Hz, 2CH_3), 1.12 (m, 6H, aliphatic-3CH_2), 0.78 (t, 3H, J = 7.2 Hz, aliphatic-CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 167.2 (C-11 and C-12, C=O), 145.8 (C-2 and C-6, C=C), 100.9 (C-3 and C-5, C=C), 58.7 (C-10 and C-13, OCH_2), 36.4 (-$\underline{CH_2}CH_2CH_2CH_3$), 31.9 (C-4, CH), 26.5 (-$CH_2\underline{CH_2}CH_2CH_3$), 22.2 (-$CH_2CH_2\underline{CH_2}CH_3$), 18.1 (C-7 and C-8, CH_3), 14.3 (C-9 and C-14, $OCH_2\underline{CH_3}$), 14.0 (-$CH_2CH_2CH_2\underline{CH_3}$); EI-MS:*m/z* (rel.

abund. %), 309 (M^+), 264 (33), 252 (100), 224 (40), 196 (49), 179 (12), 150 (8); HREI-MS: *m/z* Calcd for $C_{17}H_{27}NO_4$: 309.1940; Found: 309.1943; Anal. Calcd for $C_{17}H_{27}NO_4$ (309.40): C, 65.99; H, 8.80; N, 4.53; O, 20.68; Found:C, 66.01; H, 8.78; N, 4.56.

4.1.16. Diethyl 4-(3,4-dimethoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ***(16)***

Yield: 84%; M.p.: 145-147 °C (Lit. M.p.[22] 147 °C); R_f: 0.32 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.75 (s, 1H, NH), 6.77 (d, 1H, $J_{5',6'}$ = 8.4 Hz, H-5′), 6.72 (d, 1H, $J_{2',6'}$ = 1.5 Hz, H-2′), 6.61 (dd, 1H, $J_{6',5'}$ = 8.4 Hz, $J_{6',2'}$ = 1.5 Hz, H-6′), 4.77 (s, 1H, H-4), 3.99 (m, 4H, 2CH_2), 3.66 (s, 6H, 2CH_3), 2.23 (s, 6H, 2CH_3), 1.13 (t, 6H, *J* = 7.2 Hz, 2CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 167.0 (C-11 and C-12, C=O), 147.8 (C-4′, C-Ar), 147.0 (C-3′, C-Ar), 145.0 (C-2 and C-6, C=C), 140.9 (C-1′, C-Ar), 119.1 (C-6′, C-Ar), 111.7 (C-5′, C-Ar), 111.4 (C-2′, C-Ar), 101.9 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 55.4 (Ar-OCH_3), 55.2 (Ar-OCH_3), 38.2 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.2 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 390 (M^++1, 7.8), 389 (M^+, 30), 360 (42), 316 (56), 252 (100), 224 (57), 196 (86), 150 (18), 138 (14), 77 (12); HREI-MS: *m/z* Calcd for $C_{21}H_{27}NO_6$: 389.1838; Found: 389.1840; Anal. Calcd for $C_{21}H_{27}NO_6$ (389.44): C, 64.77; H, 6.99; N, 3.60; O, 24.65; Found: C, 64.73; H, 6.95; N, 3.58.

4.1.17. Diethyl 4-(3-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate ***(17)***

Yield: 78%; M.p.: 181-183 °C (Lit. M.p.[16] 180-182 °C); R_f: 0.27 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 9.08 (s, 1H, OH), 8.73 (s, 1H, NH), 6.95 (t, 1H, $J_{5'/4',6'}$ = 7.8 Hz, H-5′), 6.57 (br. d, 2H, $J_{4',6'/5'}$ = 7.2 Hz, H-4′/H-6′), 6.47 (dd, 1H, $J_{2',4'}$ = 1.0, $J_{2',6'}$ = 1.8 Hz, H-2′), 4.79 (s, 1H, H-4), 3.99 (m, 4H, 2CH_2), 2.23 (s, 6H, 2CH_3), 1.13 (t, 6H, *J* = 7.2 Hz, 2CH_3);

^{13}C-NMR (100 MHz, DMSO-d_6): δ 167.0 (C-11 and C-12, C=O), 156.9 (C-3′, C-Ar), 149.3 (C-1′, C-Ar), 145.0 (C-2 and C-6, C=C), 128.5 (C-5′, C-Ar), 117.9 (C-6′, C-Ar), 114.3 (C-4′, C-Ar), 112.7 (C-2′, C-Ar), 101.7 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 38.5 (C-4, CH), 18.2 (C-7 and C-8, CH_3), 14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 346 (M^++1, 4.7), 345 (M^+, 20), 272 (26), 252 (100), 224 (49), 196 (68), 150 (10); HREI-MS: *m/z* Calcd for $C_{19}H_{23}NO_5$: 345.1576; Found: 345.1582; Anal. Calcd for $C_{19}H_{23}NO_5$ (345.39): C, 66.07; H, 6.71; N, 4.06; O, 23.16; Found: C, 66.04; H, 6.69; N, 4.09.

*4.1.18. 2-[3,5-Bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydro-4-pyridinyl]benzoic acid (**18**)*

Yield: 90%; M.p.: 208-209 °C; R_f: 0.21 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 12.50 (s, 1H, COOH), 8.72 (s, 1H, NH), 7.55 (d, 1H, $J_{3',4'}$ = 7.5 Hz, H-3′), 7.37 (m, 2H, H-4′/H-6′), 7.15 (td, 1H, $J_{5',3'}$ = 1.8 Hz, $J_{5',4'}$ = $J_{5',6'}$ = 7.8 Hz, H-5′), 5.72 (s, 1H, H-4), 4.0 (m, 4H, $2CH_2$), 2.19 (s, 6H, $2CH_3$), 1.04 (t, 6H, J = 7.2 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 168.7 (-COOH), 167.3 (C-11 and C-12, C=O), 148.9 (C-2′, C-Ar), 145.0 (C-2 and C-6, C=C), 131.5 (C-4′, C-Ar), 130.3 (C-1′, C-Ar), 129.6 (C-6′, C-Ar), 128.8 (C-5′, C-Ar), 125.6 (C-3′, C-Ar), 103.0 (C-3 and C-5, C=C), 58.9 (C-10 and C-13, OCH_2), 34.9 (C-4, CH), 18.2 (C-7 and C-8, CH_3), 14.0 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 373 (M^+), 344 (69), 300 (99), 252 (50), 224 (14), 196 (43), 105 (100), 77 (51); HREI-MS: *m/z* Calcd for $C_{20}H_{23}NO_6$: 373.1525; Found: 373.1532; Anal. Calcd for $C_{20}H_{23}NO_6$ (373.40): C, 64.33; H, 6.21; N, 3.75; O, 25.71; Found: C, 64.28; H, 6.25; N, 3.72.

*4.1.19. Diethyl 2,4,6-trimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**19**)*

Yield: 92%; M.p.: 130-131 °C (Lit. M.p.[22] 130 °C); R_f: 0.64 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.62 (s, 1H, NH), 4.04 (m, 4H, $2CH_2$), 3.65 (q, 1H, H-4), 2.17

(s, 6H, $2CH_3$), 1.18 (t, 6H, J = 7.2 Hz, $2CH_3$), 0.81 (d, 3H, J = 6.6 Hz, CH_3); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 166.9 (C-11 and C-12, C=O), 145.6 (C-2 and C-6, C=C), 102.3 (C-3 and C-5, C=C), 58.8 (C-10 and C-13, OCH_2), 27.8 (C-4, CH), 22.5 (Aliphatic-CH_3), 18.1 (C-7 and C-8, CH_3), 14.3 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 267 (M^+, 2.7), 252 (100), 224 (31), 83 (16); HREI-MS: *m/z* Calcd for $C_{14}H_{21}NO_4$: 267.1471; Found: 267.1458; Anal. Calcd for $C_{14}H_{21}NO_4$ (267.32): C, 62.90; H, 7.92; N, 5.24; O, 23.94; Found:C, 62.89; H, 7.94; N, 5.27.

*4.1.20. Diethyl 2,6-dimethyl-4-propyl-1,4-dihydropyridine-3,5-dicarboxylate (**20**)*

Yield: 75%; M.p.: 122-123 °C (Lit. M.p.[19] 122 °C); R_f: 0.67 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.61 (s, 1H, NH), 4.04 (m, 4H, $2CH_2$), 3.75 (t, 1H, J = 5.1 Hz, H-4), 2.18 (s, 6H, $2CH_3$), 1.18 (t, 6H, J = 7.2 Hz, $2CH_3$), 1.12 (m, 4H, aliphatic-$2CH_2$), 0.77 (t, 3H, J = 6.6, CH_3); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 167.2 (C-11 and C-12, C=O), 145.9 (C-2 and C-6, C=C), 100.9 (C-3 and C-5, C=C), 58.8 (C-10 and C-13, OCH_2), 39.1 (-$\underline{CH_2}CH_2CH_3$), 31.8 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 17.4 (-$CH_2\underline{CH_2}CH_3$), 14.3 (C-9 and C-14, $OCH_2\underline{CH_3}$), 14.1 (-$CH_2CH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 295 (M^+), 252 (100), 224 (37), 206 (58), 196 (48), 178 (24), 150 (69); HREI-MS: *m/z* Calcd for $C_{16}H_{25}NO_4$: 295.1784 Found: 295.1764; Anal. Calcd for $C_{16}H_{25}NO_4$ (295.37): C, 65.06; H, 8.53; N, 4.74; O, 21.67; Found: C, 65.02; H, 8.48; N, 4.77.

*4.1.21. Diethyl 4-isopropyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**21**)*

Yield: 75%; M.p.: 94-96 °C (Lit. M.p.[21] 95-98 °C); R_f: 0.72 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO- d_6): δ 8.62 (s, 1H, NH), 4.02 (m, 4H, $2CH_2$), 3.73 (d, 1H, J = 5.1 Hz, H-4), 2.0 (s, 6H, $2CH_3$), 1.42 (m, 1H, CH), 1.18 (t, 6H, J = 7.2 Hz, $2CH_3$), 0.64 (d, 6H, J = 6.9

Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 167.8 (C-11 and C-12, C=O), 145.6 (C-2 and C-6, C=C), 99.4 (C-3 and C-5, C=C), 58.7 (C-10 and C-13, OCH_2), 38.0 (C-4, CH), 35.0 (-$\underline{CH}(CH_3)_2$), 18.2 (-$CH(\underline{CH_3})_2$), 18.1 (C-7 and C-8, CH_3), 14.3 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 295 (M^+), 252 (100), 224 (68), 196 (94), 179 (23), 150 (20); HREI-MS: *m/z* Calcd for $C_{16}H_{25}NO_4$: 295.1784; Found: 295.1778; Anal. Calcd for $C_{16}H_{25}NO_4$ (295.37): C, 65.06; H, 8.53; N, 4.74; O, 21.67; Found: C, 65.03; H, 8.51; N, 4.78.

*4.1.22. Diethyl 4-isobutyl-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**22**)*

Yield: 72%; M.p.: 82-84 °C; R_f: 0.75 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.70 (s, 1H, NH), 4.02 (m, 4H, $2CH_2$), 3.78 (t, 1H, J = 6.6 Hz, H-4), 2.18 (s, 6H, $2CH_3$), 1.39 (m, 1H, CH), 1.18 (t, 6H, J = 7.2 Hz, $2CH_3$), 0.97 (t, 2H, J = 6.6 Hz, CH_2), 0.80 (d, 6H, J = 6.6 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 167.2 (C-11 and C-12, C=O), 145.7 (C-2 and C-6, C=C), 101.8 (C-3 and C-5, C=C), 58.8 (C-10 and C-13, OCH_2), 47.1 (-$\underline{CH_2}CH(CH_3)_2$), 30.0 (C-4, CH), 23.1 (-$CH_2\underline{CH}(CH_3)_2$), 22.9 (-$CH_2CH(\underline{CH_3})_2$), 18.1 (C-7 and C-8, CH_3), 14.2 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 309 (M^+), 262 (35), 252 (84), 206 (100), 195 (24), 178 (51), 150 (22), 106 (16); HREI-MS: *m/z* Calcd for $C_{17}H_{27}NO_4$: 309.1940; Found: 309.1923; Anal. Calcd for $C_{17}H_{27}NO_4$ (309.40): C, 65.99; H, 8.80; N, 4.53; O, 20.68; Found: C, 65.95; H, 8.77; N, 4.51.

*4.1.23. Diethyl 4-(3,4-dihydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (**23**)*

Yield: 78%; M.p.:149-150 °C (Lit. M.p.[24] 147-151 °C); R_f: 0.21 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.66 (s, 1H, 3′-OH), 8.58 (s, 1H, 4′-OH), 8.49 (s, 1H, NH), 6.54 (d, 1H, $J_{2',6'}$ = 2.0 Hz, H-2′), 6.50 (d, 1H, $J_{5',6'}$ = 8.0 Hz, H-5′), 6.39 (dd, 1H, $J_{6',2'}$ = 2.0 Hz, $J_{6',5'}$ = 8.0 Hz, H-6′), 4.68 (s, 1H, H-4), 3.97 (m, 4H, $2CH_2$), 2.21 (s, 6H, $2CH_3$), 1.13 (t, 6H, J =

7.0 Hz, $2CH_3$); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 167.1 (C-11 and C-12, C=O), 144.5 (C-2 and C-6, C=C), 144.4 (C-4′, C-Ar), 143.3 (C-3′, C-Ar), 139.3 (C-1′, C-Ar), 118.0 (C-6′, C-Ar), 114.9 (C-5′, C-Ar), 114.8 (C-2′, C-Ar), 102.2 (C-3 and C-5, C=C), 58.8 (C-10 and C-13, OCH_2), 37.8 (C-4, CH), 18.1 (C-7 and C-8, CH_3), 14.2 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 362 (M^++1, 2.2), 361 (M^+, 10.7), 332 (15), 288 (27), 252 (100), 224 (19), 196 (32), 150 (7); HREI-MS: *m/z* Calcd for $C_{19}H_{23}NO_6$: 361.1525; Found: 361.1520; Anal. Calcd for $C_{19}H_{23}NO_6$ (361.39): C, 63.15; H, 6.41; N, 3.88; O, 26.56; Found: C, 63.12; H, 6.39; N, 3.83.

*4.1.24. Diethyl 2,6-dimethyl-4-phenethyl-1,4-dihydropyridine-3,5-dicarboxylate (**24**)*

Yield: 84%; M.p.: 104-105 °C; R_f: 0.70 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.71 (s, 1H, NH), 7.20 (t, 2H, $J_{3'/2',4'} = J_{5'/4',6'} =$ 7.5 Hz, H-3′/5′), 7.10 (t, 1H, $J_{4'/3',5'} =$ 7.5 Hz, H-4′), 7.06 (br. d, 2H, $J_{2',3'} = J_{6',5'} =$ 7.5 Hz, H-2′/H-6′), 4.06 (m, 4H, $2CH_2$), 3.87 (t, 1H, J = 6.0 Hz, H-4), 2.40 (m, 2H, CH_2), 2.20 (s, 6H, $2CH_3$), 1.48 (m, 2H, CH_2), 1.17 (t, 6H, J = 7.5 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 167.1 (C-11 and C-12, C=O), 146.3 (C-2 and C-6, C=C), 142.5 (C-1′, C-Ar), 128.1 (C-3′ and C-5′, C-Ar), 127.9 (C-2′ and C-6′, C-Ar), 125.3 (C-4′, C-Ar), 100.5 (C-3 and C-5, C=C), 58.8 (C-10 and C-13, OCH_2), 38.2 (-$CH_2\underline{CH_2}$Ph), 32.4 (C-4, CH), 30.5 (-$\underline{CH_2}CH_2$Ph), 18.2 (C-7 and C-8, CH_3), 14.3 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 357 (M^+), 312 (16), 252 (100), 224 (39), 196 (55), 150 (11), 105 (12), 91(13); HREI-MS: *m/z* Calcd for $C_{21}H_{27}NO_4$:357.1940; Found: 357.1953; Anal. Calcd for $C_{21}H_{27}NO_4$ (357.44): C, 70.56; H, 7.61; N, 3.92; O, 17.90; Found: C, 70.61; H, 7.59; N, 3.88.

*4.1.25. Diethyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**25**)*

Yield: 95%; M.p.: 131-133 °C (Lit. M.p.[24] 128-132 °C); R_f: 0.51 (*n*-hexane/ethyl acetate, 8:2); ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 8.96 (s, 1H, NH), 8.09 (d, 2H, $J_{3',2'} = J_{5',6'} =$ 8.5 Hz, H-3′/H-5′),

7.39 (d, 2H, $J_{2',3'}$= $J_{6',5'}$ =8.5 Hz, 2H, H-2′/H-6′), 4.95 (s, 1H, H-4), 3.97 (m, 4H, $2CH_2$), 2.26 (s, 6H, $2CH_3$), 1.11 (t, 6H, J = 7.5 Hz, $2CH_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 166.5 (C-11 and C-12, C=O), 155.4 (C-4′, C-Ar), 146.2 (C-2 and C-6, C=C), 145.8 (C-1′, C-Ar), 128.5 (C-3′ and C-5′, C-Ar), 123.2 (C-2′ and C-6′, C-Ar), 100.8 (C-3 and C-5, C=C), 59.2 (C-10 and C-13, OCH_2), 39.4 (C-4, CH), 18.2 (C-7 and C-8, CH_3), 14.1 (C-9 and C-14, $OCH_2\underline{CH_3}$); EI-MS: *m/z* (rel. abund. %), 375 (M^++1, 4.6), 374 (M^+, 20), 345 (42), 329 (42), 301 (43), 252 (100), 224 (52), 196 (78), 150 (14); HREI-MS: *m/z* Calcd for $C_{19}H_{22}N_2O_6$:374.1478; Found: 374.1497; Anal. Calcd for $C_{19}H_{22}N_2O_6$ (374.39): C, 60.95; H, 5.92; N, 7.48; O, 25.64; Found: C, 60.91; H, 5.89; N, 7.51.

4.2. In vitro α-glucosidase inhibition assay

α-Glucosidase (EC 3.2.1.20, *Saccharomyces cerevisiae*, Sigma catalog # G0660) inhibitory activity was assayed by using 0.1 M phosphate buffer (pH 6.8) at 37 °C [35]. The enzyme (0.2 U/mL) in phosphate buffer saline was incubated with various concentrations of test compounds at 37 °C for 15 min, test compounds were dissolved in DMSO (final concentration 7%). The substrate and *p*-nitrophenyl *α*-D-glucopyranoside (0.7 mM, final) were added and change in absorbance at 400 nm was monitored up to 30 min. Test compound was replaced by DMSO (7% final) as control. Acarbose was used as the standard inhibitor. The percent inhibition was calculated by using the following formula:

$$\% \text{ Inhibition} = 100 - (\text{OD test well}/\text{OD control}) \times 100$$

4.3. Cytotoxicity evaluation of compounds on rat fibroblast 3T3 cell lines

The experiment was performed according to the method described by Dimas *et al*. [34]. Rat fibroblast 3T3 cells were used in this assay. Briefly the 3T3-adherent cells (2×105 cells/mL) were cultured in a 96-well plate overnight in a CO_2 environment at 37 °C. Supernatant was removed and 50 μL of serially diluted compounds (100 - 12.5 μg/mL) and 150 μL complete medium (DMEM supplemented with 5% (v/v) fetal bovine serum, penicillin (100 units/mL) and streptomycin (100 μg/mL) were added to each well. After the incubation, the culture medium was aspirated carefully and 50 μL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) solution (2 mg/mL in PBS) was added to each well and further incubated for 4 h. After this, MTT solution was aspirated and cells were PBS-washed once, and 100 μL of DMSO was added to dissolve the blue insoluble MTT formazan produced by the action of mitochondrial dehydrogenase. The plate was agitated at room temperature for 15 minutes and then read at 540 nm by using microplate readers (SpectraMax Plus-384). The percentage of viable cells was calculated as the relative ratio of optical densities.

4.4. In vitro carbonic anhydrase-II inhibition assay

In this assay, 4-nitrophenyl acetate (4-NPA), which is colorless, is hydrolyzed to 4-nitrophenol and CO_2 and reaction is followed by measuring the formation of 4-nitrophenol, a yellow colored compound. The experiment was performed in buffer containing HEPES and Tris at a total concentration of 20 mM and pH was 7.4. For each sample the reaction tube contained 140 μL of the HEPES-*Tris* solution, 20 μL of freshly prepared aqueous solution of purified bovine erythrocyte CA-II (0.1 mg/mL of deionized water for 96-well), 20 μL of test compound dissolve in DMSO (10% final concentration), 20 μL of substrate 4-PNA at concentration of 0.7

mM diluted in ethanol. The reaction was initiated by addition of 4-PNA after 15 min. incubation of test compound. The compounds were tested for 3-times at different concentration. In this assay the reaction was performed in 96-well plate. SPECTRA max 340 spectrophotometer, Molecular devices (USA) was used to monitor the reaction and the amount of product formed was monitored at 1 min interval for 30min at 400 nm [36].

4.5. In vitro phosphodiesterase-I inhibition assay

Activity against snake venom was assayed by taking 33 mM Tris–HC1 buffer pH 8.8, 30 mM Mg-acetate with 0.000742 U/well final concentration of enzyme using a microtiter plate assay and 0.33 mM *bis*-(*p*-nitropheny1) phosphate (Sigma N-3002) as substrate. From Merck Cystein and EDTA were used as positive controls ($1C_{50}$ = 748 μM ± 0.015, 274 μM ± 0.007, respectively).

After 30 min pre-incubation of the enzyme with the test samples, enzyme activity was monitored spectrophotometrically at 37 $^{\circ}$C on a microtitre plate reader (SpectraMax, Molecular Devices) by following the rate (change in O.D/min) of release of *p*-nitrophenol from p-nitrophenyl phosphate at 410 nm. All assays were conducted in triplicate [37].

4.6. In vitro β-Glucuronidase inhibition assay

β-Glucuronidase activity was determined by means of the spectrophotometer by measuring the absorbance at 405 nm of *p*-nitrophenol produced from the substrate. The entire reaction volume was 250 μL. The reaction mixture contained 185 μL of 0.1 M acetate buffer, 5 μL of test compound solution, 10 μL of (1U) enzyme solution was incubated at 37 $^{\circ}$C for 30 min. The test compound was dissolve in DMSO, from which 5 μL was added to per well (, which become 2%

in the ultimate assay and the similar conditions were used for standard (D-saccharic acid 1,4-lactone). The plates were read on a multiplate reader (SpectraMax plus 384) after the addition of 50 μL of 0.4 mM *p*-nitrophenyl-β-D-glucuronide. All assays were run in triplicate [38].

4.7. Statistical analysis

The EZ-Fit Enzyme Kinetics program (Perrella Scientific Inc., Amherst, USA) were employed to calculate the IC_{50} values. All graphs were plotted by using GraFit program (1999). Values of the correlation coefficients, intercepts, slopes, and their standard errors were calculated by the linear regression analysis by using the same program. Each point in the constructed graphs represents the mean of the three experiments [39].

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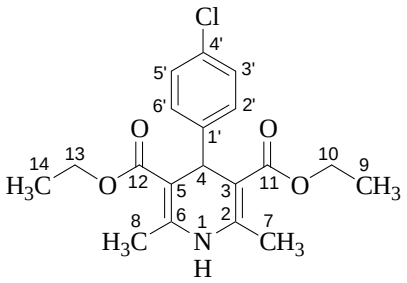
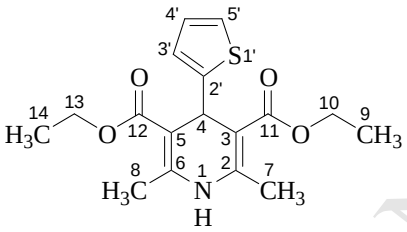
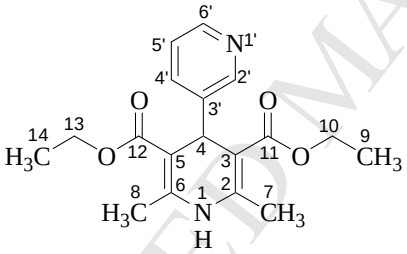
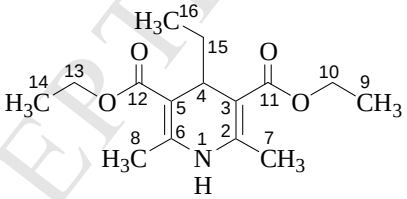
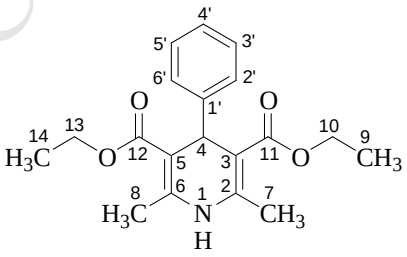
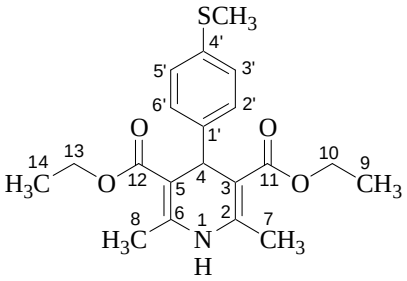
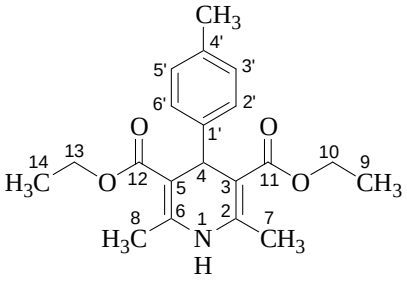
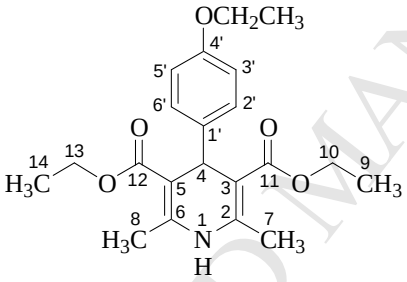
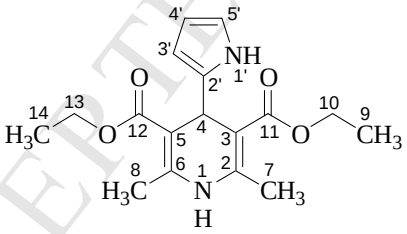
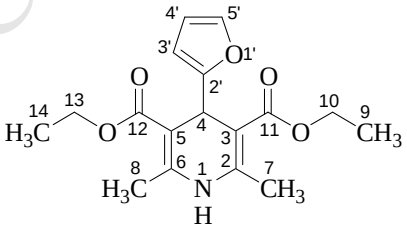
Legends to Figures:

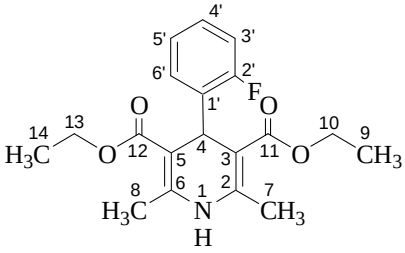
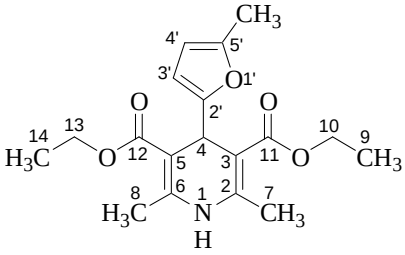
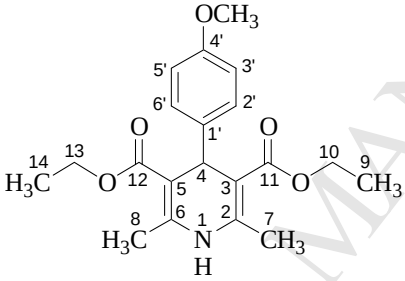
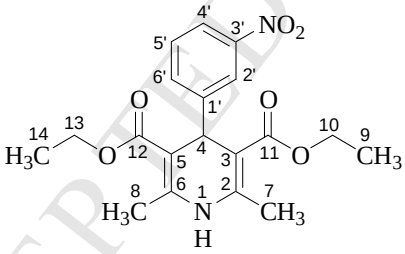
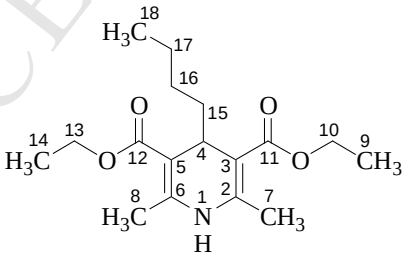
Figure-2: The inhibition of α-glucosidase by compound **14** (A) is the Lineweaver-Burk plot of reciprocal of rate of reaction (velocities) *versus* reciprocal of substrate in the absence (▲), and in presence of 25 μM (Δ), 30 μM (■), 35μM (□), 40 μM (●) and 50 μM (○) of compound **14**. The figure (B) is the secondary replot of Lineweaver-Burk plot between the slopes of each line on Lineweaver-Burk plot *versus* different concentrations of compound **14**. (C) is the Dixon plot of reciprocal of rate of reaction (velocities) *versus* different concentrations of compound **14**.

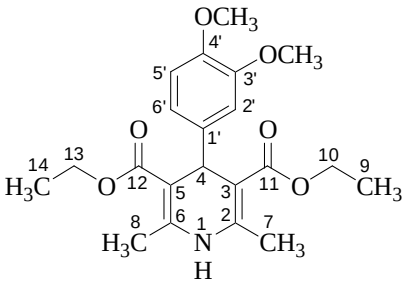
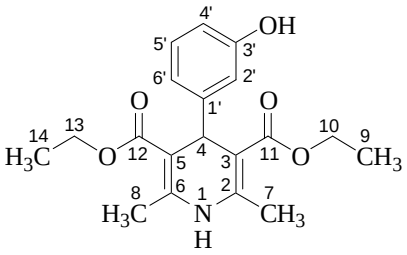
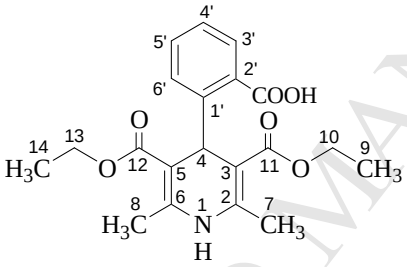
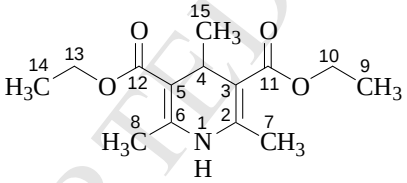
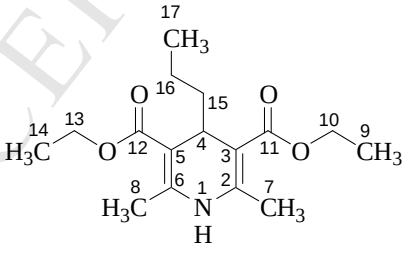
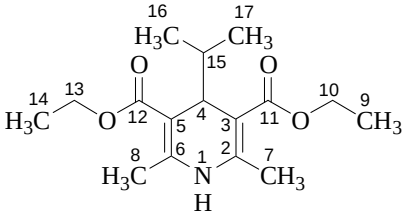
Figure-3: The inhibition of α-glucosidase by compound **25** (A) is the Lineweaver-Burk plot of reciprocal of rate of reaction (velocities) *versus* reciprocal of substrate in the absence (▲), and in presence of 60 μM (□), 70 μM (●) and 80 μM (○) of compound **25**. The figure (B) is the secondary replot of Lineweaver-Burk plot between the slopes of each line on Lineweaver-Burk plot *versus* different concentrations of compound **25**. (C) is the Dixon plot of reciprocal of rate of reaction (velocities) *versus* different concentrations of compound **25**.

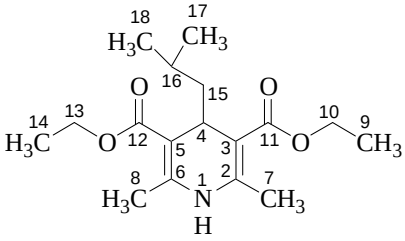
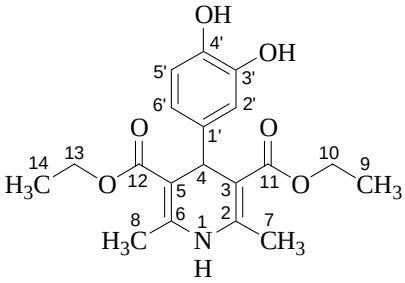
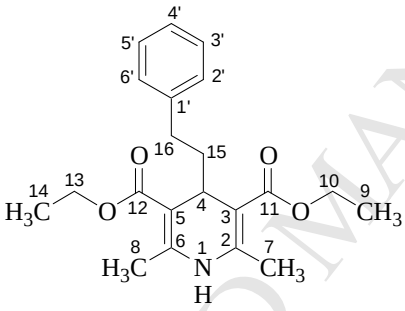
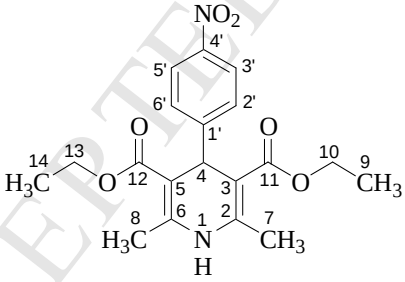
Table-1: *α*-Glucosidase inhibitiory activity of compounds **1-25**.

Compound	Product	IC_{50} (μM ± SEM[a])
1		72.4 ± 1.03
2		74.3 ± 0.76
3		[b]NA
4		[b]NA
5		[b]NA

6		97.4 ± 0.32
7		117.5 ± 1.81
8		124.3 ± 0.97
9		[b]NA
10		[b]NA

11		60.2 ± 1.01
12		[b]NA
13		65.1 ± 0.92
14		35.0 ± 0.17
15		122.0 ± 0.91

16		[b]NA
17		[b]NA
18		[b]NA
19		[b]NA
20		[b]NA
21		[b]NA

22		[b]NA
23		273.7 ± 1.04
24		112.0 ± 0.64
25		51.0 ± 0.98
Standard	Acarbose	937 ± 1.60

[a]S.E.M: Standard error mean; [b]NA: Not active

Table-2: Carbonic anhydrase-II, phosphodiestrase-I and *β*-glucuronidase inhibition of compounds **2**, **13**, **14**, and **25**.

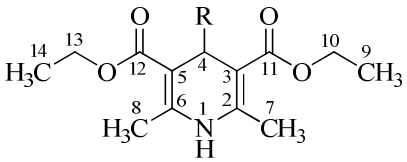
Compounds	Carbonic anhydrase-II		Phosphodiestrase-I		*β*-Glucuronidase	
	% Inhibition	IC_{50} ± [a]SEM (*μ*M)	% Inhibition	IC_{50} ± [a]SEM (*μ*M)	% Inhibition	IC_{50} ± [a]SEM (*μ*M)
2	Negative	[b]NA	8.0	[b]NA	Negative	[b]NA
13	Negative	[b]NA	8.1	[b]NA	12.4	[b]NA
14	11.7	[b]NA	11.4	[b]NA	22.3	[b]NA
25	9.7	[b]NA	20.3	[b]NA	14.1	[b]NA

[a]S.E.M: Standard error mean; [b]NA: Not active

Table-3: Cytotoxicity of active compounds **1**, **2**, **6-8**, **11**, **13**, **14**, and **23-25** against 3T3 cell line.

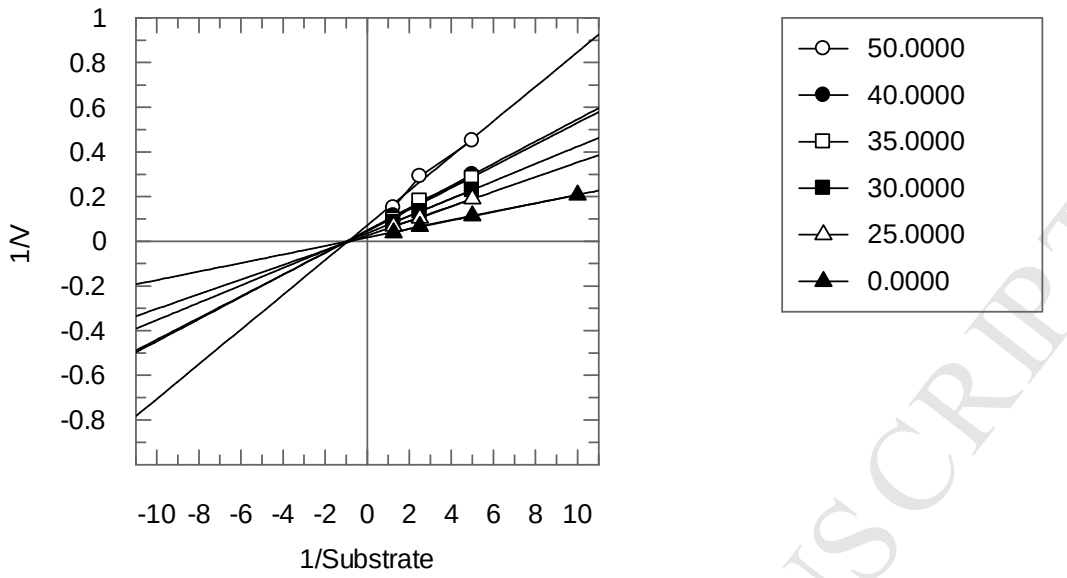
Compounds	**Cytotoxicity (3T3 Cell line) IC_{50} ± [a]SEM (μM)**
1	27.86 ± 0.25
2	>30
6	>30
7	>30
8	>30
11	>30
13	>30
14	>30
23	28.63 ± 0.24
24	26.73 ± 0.52
25	>30

[a]SEM: Standard error mean

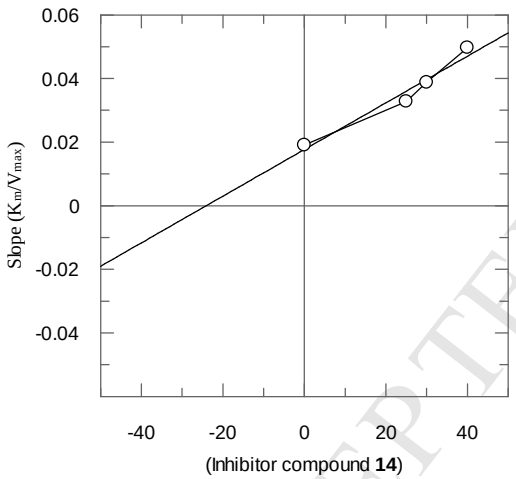


1-25

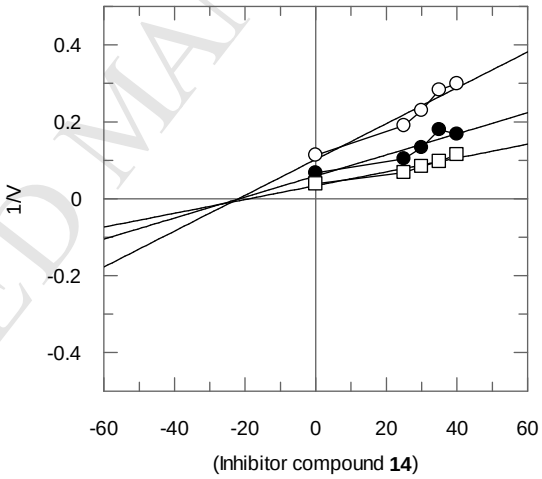
Figure-1



A

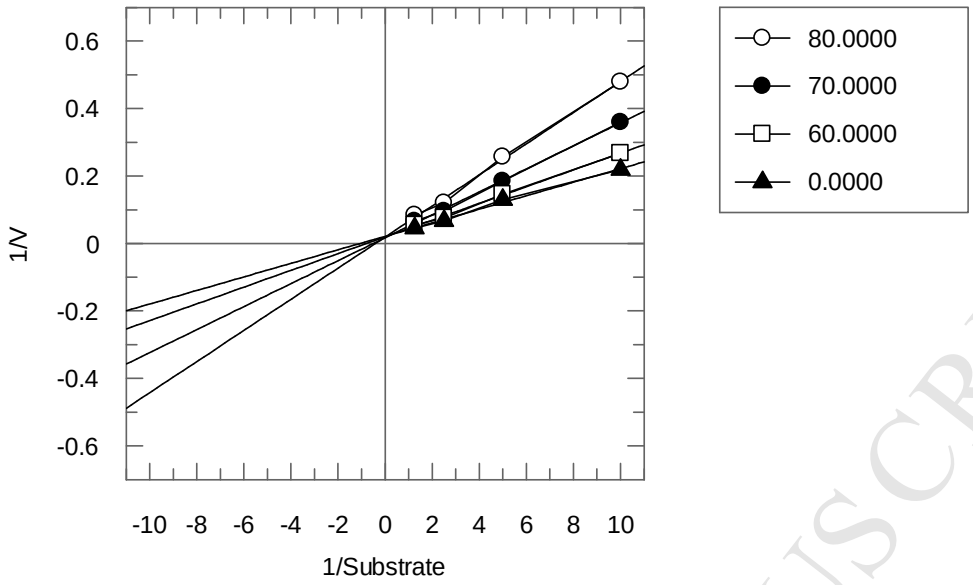


B

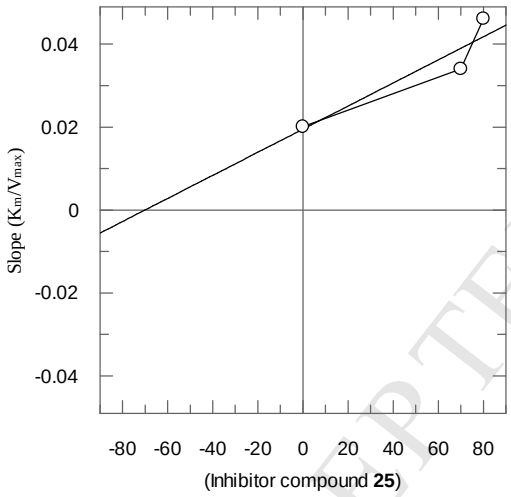


C

Figure-2:



A



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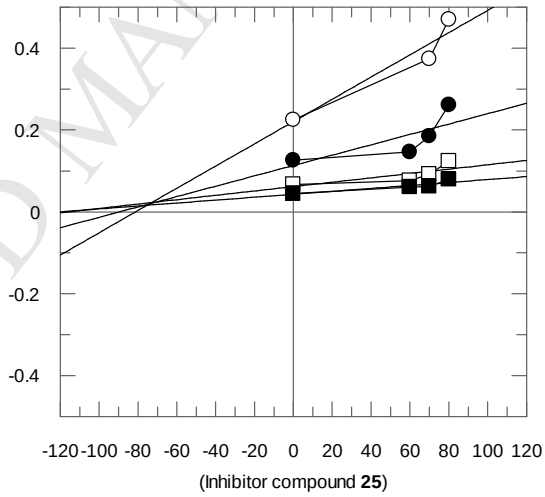

C

Figure-3:

Highlights (for review)

- Synthesis of 1,4-Dihydropyridine-3,5-dicarboxylates derivatives
- α-Glucosidase inhibitory activities
- Anti-hyperglycemic activity
- Kinetic studies
- Competitive and non-competitive inhibitors