



New Gemcitabine Derivatives as Potent in vitro α -Glucosidase Inhibitors

Farked Wahoodi Salman¹, Ahmed Jasim Twayej², Hayder Ahmed Shaheed³, Ali Jabbar Radhi³✉

¹Department of Chemistry, Faculty of Science, Kufa University, Najaf-Iraq.

²Faculty of Health and Medical Techniques, Al-Kafeel University, Najaf, Iraq.

³Faculty of Pharmacy, Al-Kafeel University, Najaf, Iraq.

✉ Corresponding author. E-mail: Alijebar56@gmail.com

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Abstract

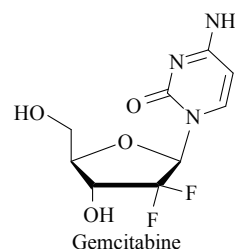
In this work, new heterocyclic compounds 1,2,3-triazoline derivatives starting from gemcitabine were synthesized. At first, gemcitabine was converted to 2-azido gemcitabine (G) through the reaction of gemcitabine with sodium azide. 1,2,3-Triazolines were prepared from the reaction of 2-azido gemcitabine with some unsaturated compounds such as malic anhydride, cinamic acid and acryl amide by click reaction. The products were identified by Fourier-transform infrared spectroscopy (FTIR) and proton nuclear magnetic resonance (¹H-NMR) technique. The α -glucosidase inhibitory activities of all the synthesized compounds were determined in vitro. All the tested compounds showed α -glucosidase inhibitory activity of $IC_{50} = 144.8 \pm 1.74$, 212.9 ± 3.4 and 345 ± 4.5 μ M against the α -glucosidase enzyme when compared to the standard drug acarbose $IC_{50} = 824 \pm 1.73$ μ M.

Keywords: Gemcitabine; 1,2,3-triazolines; α -glucosidase inhibitors

Introduction

Alpha-glucosidase is an enzyme that catalyzes the hydrolysis of polysaccharides into monosaccharide, leading to post-prandial hyperglycemia in diabetic patients [1]. This high blood glucose level (hyperglycemia) causes damages to vital organs, and leads to complications, such as cataract, retinopathy, neuropathy, atherosclerosis, nephropathy and impaired wound healing [2-5]. α -Glucosidase inhibition has been reported mainly to overcome the risk of hyperglycemia after eating in diabetics, which in turn is associated with the above-mentioned health disorders [6,7]. Acarbose, 1-dioxinogermesin and miglitol were developed as α -glucosidase inhibitors. However, many

of them have adverse effects and low patient tolerance. Gemcitabine (Scheme 1), a pyrimidine fluorinated nucleoside analog, is chemically known as 1-(2'-deoxy-2',2'-difluoro-D-ribofuranosyl)-4-aminopyrimidin-2-one hydrochloride and 2',2'-difluorodeoxycytidine. Originally developed by Lilly, it is an anticancer drug



Scheme 1 Structural formula of gemcitabine.

marketed as the HCl salt under the trade name of Gemzar (Lilly) [8]. Gemcitabine structure is similar to cytarabine; it has a wider spectrum of solid antitumor activity because of its difference in pharmacology and the mechanism of action [9]. Gemcitabine is a type of chemotherapy to treat many types of cancer including pancreatic cancer, lung cancer, ovarian cancer and breast cancer [10-12]. In pancreatic cancer, gemcitabine is administered as a single agent, but in lung cancer non-cell lung cancer and bladder cancer, it is given in conjunction with cisplatin [13]. Gemcitabine can interfere speedily with the growing cells such as cancer cells and kill this cell. Gemcitabine is a prodrug [14] which undergoes phosphorylation intracellular into the form of active triphosphate and diphosphate, with the effect of DNA synthesis leading to apoptosis [15]. In this study, a series compounds of pharmacologically active 1,2,3-triazolines derivatives based on gemcitabine were successfully prepared and their in-vitro α -glucosidase inhibitory activity was investigated.

Experimental

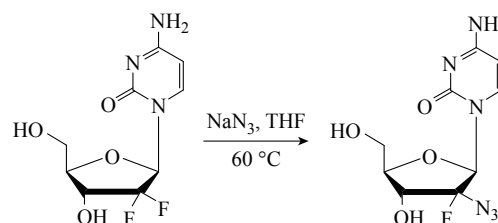
All chemicals and solvents were obtained from Merck, BDH, Fluke, and Sigma-Aldrich and were used without further purification. Fourier-transform infrared spectroscopy (FTIR) spectra were recorded by using Fourier transformation infrared Shimadzu IR-408" at Faculty of Pharmacy, University of Kufa. Elemental analysis was performed using a Perkin-Elmer 204E Instrument. Proton nuclear magnetic resonance (^1H -NMR) spectra were recorded on Bruker 300 MHz. Thin layer chromatography (TLC) was performed on a silica gel SG-40 (Merck) and developed with the solvents mentioned; spots were visualized with Iodine vapor.

Synthesis methods

General procedure for synthesis of 2-azido gemcitabine (G)

60 mmol of gemcitabine and 60 mmol of sodium azide were dissolved in 30 mL of tetrahydrofuran (THF). The resulting mixture was stirred at 60 °C for 4 h. Then, the TLC showed that the reaction was completed by using (ethyl acetate: petroleum ether, 1 : 4). Then, it was extracted with 40 mL of diethyl ether three times and then the organic phase was treated with 100 mL of water (three times). Subsequently, the diethyl ether phase was dried by adding anhydrous

magnesium sulfate MgSO_4 and then filtered. The diethyl ether was evaporated and the residue was dried and purified by recrystallization from ethanol (Scheme 2).



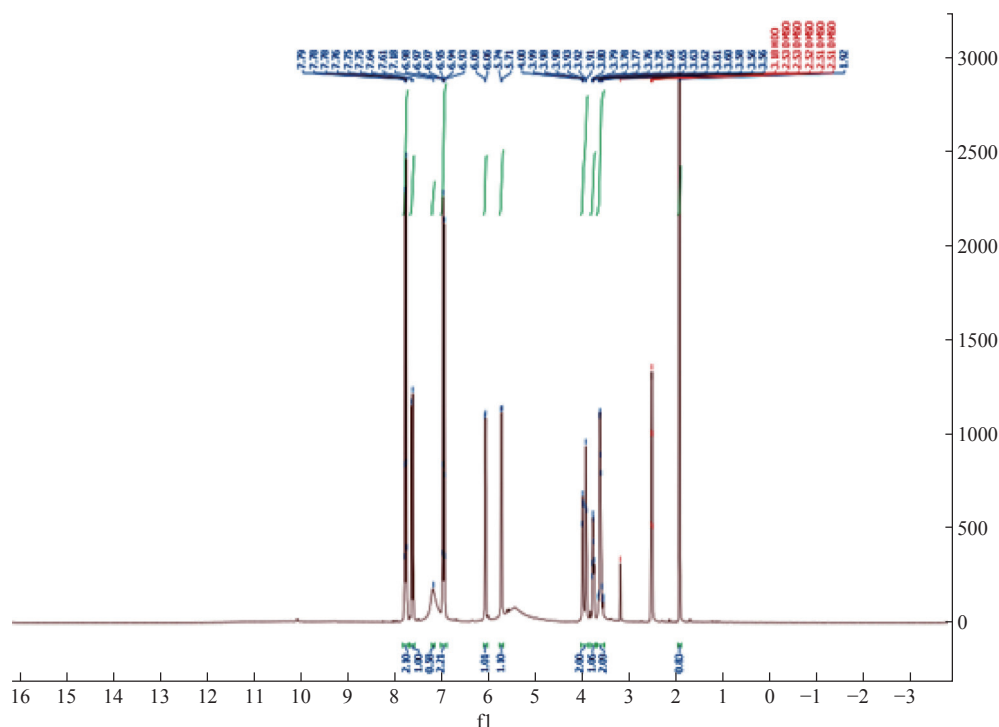
Scheme 2 Synthesis of 2-azido gemcitabine (G).

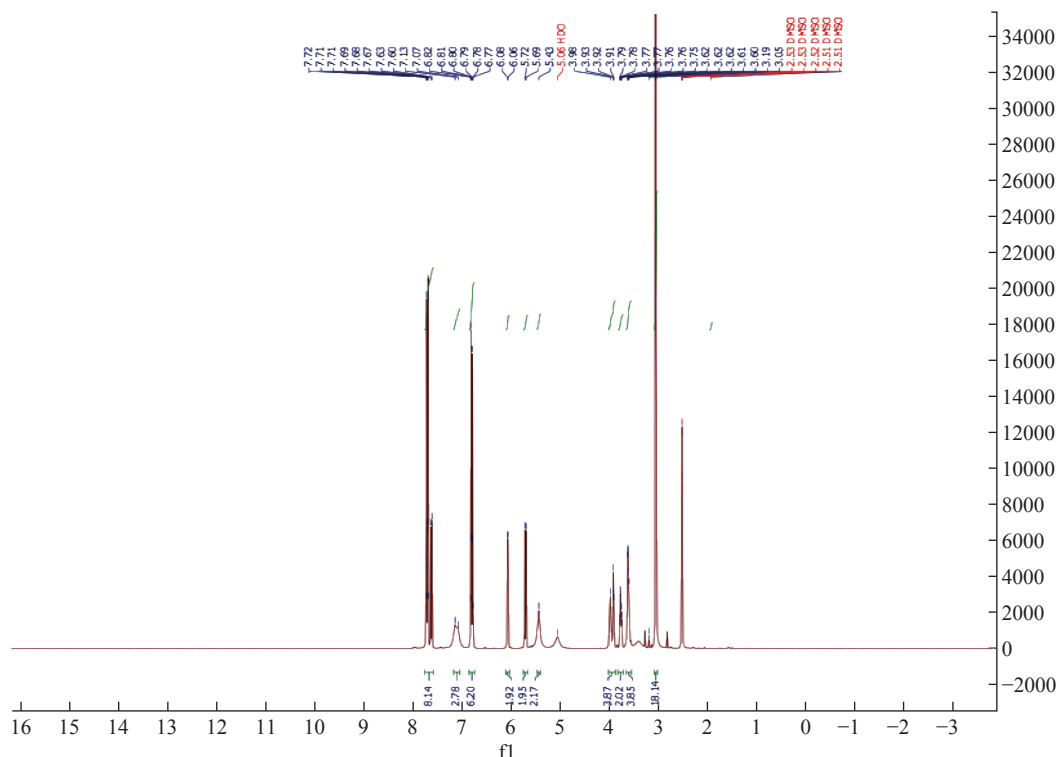
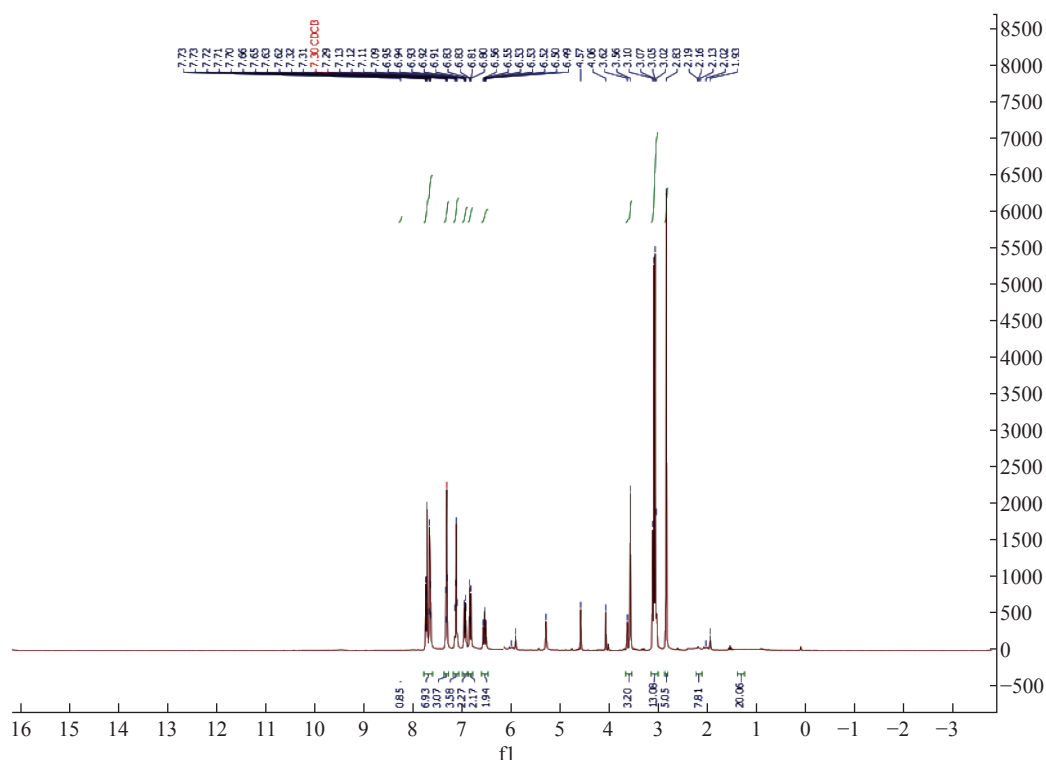
2-azido gemcitabine (G) was prepared as a white solid powder, Chemical formula: $\text{C}_9\text{H}_{11}\text{FN}_6\text{O}_4$; yield: 64%; m.p.: 240-242 °C. FTIR: 3454 cm^{-1} , 3411 cm^{-1} due to OH stretching, 2971 cm^{-1} , 2870 cm^{-1} due to CH stretching, 2105 cm^{-1} due to azide group stretching, 1671 cm^{-1} due to carbonyl group stretching, 1580 cm^{-1} due to C=N stretching, 1276 cm^{-1} , 1119 cm^{-1} , 1075 cm^{-1} due to C-O stretching and 796 cm^{-1} due to C-H bonding. ^1H -NMR (301 MHz, DMSO- d_6) (Fig. 1): δ 9.79 ppm (s, 1H), 7.83-7.71 ppm (m, 2H), 7.63 ppm (d, J = 7.5 Hz, 1H), 7.18 ppm (s, 1H), 7.01-6.90 ppm (m, 2H), 6.07 ppm (d, J = 4.0 Hz, 1H), 5.72 ppm (d, J = 7.4 Hz, 1H), 4.03-3.88 ppm (m, 2H), 3.77 ppm (td, J = 5.2, 3.1 Hz, 1H), 3.70-3.53 ppm (m, 2H), 1.92 ppm (s, 1H). Elemental analysis: calc. C, 37.77; H, 3.87; N, 29.36; Found: C, 37.01; H, 3.81; N, 29.11.

Synthesis of 1,2,3-triazoline derivatives (G1-G3)

1.1 mmol of 2-azido gemcitabine (G) and 1.1 mmol of unsaturated compounds (maleic anhydride, acrylamide and cinamic acid) were dissolved in 20 mL of ethanol. To this mixture was added sodium ascorbate (0.4 mmol) and CuCl (0.2 mmol). The solution was stirred at 60 °C until TLC indicated the reaction was completed and the consumption of 2-azido gemcitabine compound. The mixture was diluted with organic solvent (diethyl ether) and water. The organic phase was separated, and the water phase was extracted two times with diethyl ether. The organic phase was dried over MgSO_4 . The solvent was removed and recrystallized from hexane-chloroform (Scheme 3).

2-(1,2,3-triazoline-4,6-dione-1-yl) gemcitabine (G1) was prepared as a white solid. Chemical formula: $\text{C}_{13}\text{H}_{13}\text{FN}_6\text{O}_7$; reaction yield: 86%; m.p.: 182-184 °C. FTIR: 3476 cm^{-1} , 3441 cm^{-1} due to OH stretching, 3128 cm^{-1} due to CH aromatic stretching, 2938 cm^{-1} , 2823



Fig. 2 ^1H -NMR of compound G1.Fig. 3 ^1H -NMR of compound G2.

(s, 3H), 7.76-7.57 ppm (m, 8H), 7.10 ppm (d, $J = 18.5$ Hz, 3H), 6.85-6.74 ppm (m, 6H), 6.07 ppm (d, $J = 4.0$ Hz, 2H), 5.70 ppm (d, $J = 7.4$ Hz, 2H), 5.43 ppm (s, 2H), 4.01-3.88 ppm (m, 4H), 3.77 ppm (td, $J = 5.2, 3.0$ Hz, 2H), 3.66-3.55 ppm (m, 4H), 3.05 ppm (s, 18H),

1.93 ppm (s, 1H). Elemental analysis: calc.: C, 40.63; H, 3.4; N, 21.87; found: C, 40.01; H, 3.35; N, 21.77.

2-(1,2,3-triazoline-5-carboxamide-1-yl) gemcitabine (G2) was prepared as a white solid. Chemical formula: $\text{C}_{12}\text{H}_{16}\text{N}_7\text{O}_5$, reaction yield: 81%; m.p.: 202-204 °C. FTIR:

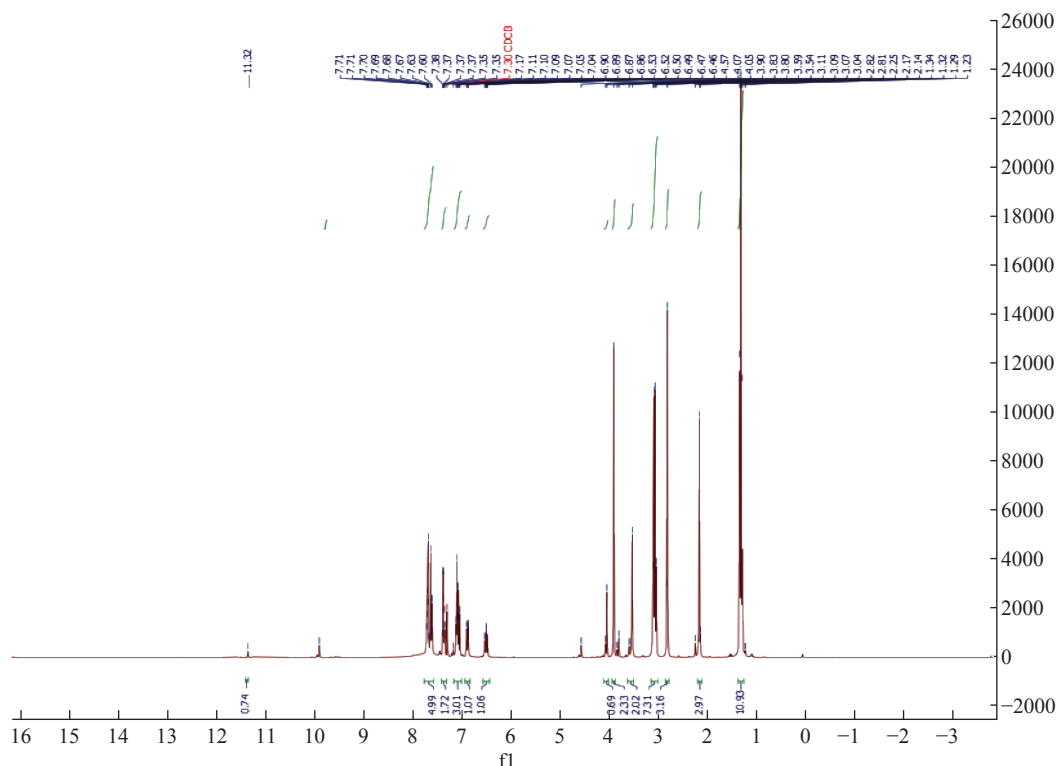


Fig. 4 ^1H -NMR of compound G3.

3471 cm^{-1} , 3440 cm^{-1} due to OH stretching, 3108 cm^{-1} due to CH aromatic stretching, 2944 cm^{-1} , 2834 cm^{-1} due to CH stretching, 1666 cm^{-1} due to carbonyl group stretching, 1622 cm^{-1} due to benzene ring stretching, 1583 cm^{-1} , 1537 cm^{-1} , 1473 cm^{-1} due to C=N and C=C stretching, 1287 cm^{-1} , 1113 cm^{-1} , 1070 cm^{-1} due to C-O stretching and 797 cm^{-1} due to C-H bonding. ^1H -NMR (301 MHz, chloroform- d): δ 8.24 ppm (s, 1H), 7.77-7.59 ppm (m, 7H), 7.35-7.25 ppm (m, 3H), 7.17-7.06 ppm (m, 4H), 6.93 ppm (dt, J = 8.9, 2.4 Hz, 2H), 6.87-6.77 ppm (m, 2H), 6.59-6.46 ppm (m, 2H), 3.59 ppm (d, J = 18.7 Hz, 3H), 3.06 ppm (q, J = 7.3 Hz, 13H), 2.83 ppm (s, 5H), 2.16 ppm (d, J = 18.0 Hz, 8H), 1.32 ppm (t, J = 7.3 Hz, 20H). Elemental analysis: calc.: C, 40.34; H, 4.51; N, 27.44; found: C, 40.31; H, 4.48; N, 27.36.

2-(4-phenyl-4,5-dihydro-1,2,3-triazole-5-carboxylic acid-1-yl)gemcitabine (G3) was prepared as a light white solid. Chemical Formula: $\text{C}_{18}\text{H}_{19}\text{FN}_6\text{O}_6$; reaction yield: 80%; m.p.: 212-214 $^{\circ}\text{C}$. FTIR: 3469 cm^{-1} , 3417 cm^{-1} due to OH stretching, 3130 cm^{-1} due to CH aromatic stretching, 2960 cm^{-1} , 2374 cm^{-1} due to CH stretching, 1671 cm^{-1} due to carbonyl group stretching, 1638 cm^{-1} due to benzene ring stretching, 1580 cm^{-1} , 1539 cm^{-1} , 1477 cm^{-1} due to C=N and C=C stretching, 1276 cm^{-1} , 1119 cm^{-1} , 1070 cm^{-1} due to C-O stretching and 798 cm^{-1} due to C-H bonding. ^1H -NMR (301 MHz, chloroform- d): δ 9.77 ppm (s, 1H), 7.76-7.57 ppm (m,

5H), 7.41-7.31 ppm (m, 2H), 7.16-7.01 ppm (m, 3H), 6.88 ppm (dd, J = 9.0, 2.5 Hz, 1H), 6.50 ppm (td, J = 8.8, 2.4 Hz, 1H), 4.06 ppm (d, J = 7.8 Hz, 1H), 3.90 ppm (s, 2H), 3.54 ppm (s, 2H), 3.08 ppm (q, J = 7.3 Hz, 7H), 2.81 ppm (s, 3H), 2.17 ppm (s, 3H), 1.32 ppm (t, J = 7.3 Hz, 11H). Elemental analysis: calc.: C, 49.77; H, 4.41; N, 19.35; found: C, 49.61; H, 4.38; N, 19.29.

Alpha-glucosidase inhibition analysis [16]

All synthesized 1,2,3-triazoline derivatives (G1-G3) were determined of their in-vitro α -glucosidase enzyme inhibitory activity. Acarbose was used as positive control drug and substrate (p-nitrophenyl glucopyranoside). A reaction mixture consisting of 30 μL 0.5 mM test compounds (G1-G3), 140 μL 50 mM phosphate buffer of 7.2 pH and 30 μL enzyme of 0.02 units. These contents were pre-incubated for 10 minutes at 37 $^{\circ}\text{C}$. The reaction was started by the addition of substrate p-nitrophenyl glucopyranoside (25 μL , 0.5 μM). After 30 minutes of incubation, the absorbance of yellow color produced due to the formation of p-nitrophenyl was measured at 405 nm. The enzymatic reaction was stopped by adding 100 μL of 200 μM Na_2CO_3 . IC_{50} values were calculated and inhibition percentage for each compound was

calculated by using the following formula:

$$\% \text{ Inhibition} = (\text{Abs control} - \text{Abs sample}) / \text{Abs control} \times 100\%$$

Results and Discussion

In this article, synthesis of new 1,2,3-triazoline derivatives was achieved from gemcitabine which was converted to 2-azido gemcitabine and by the reaction of gemcitabine and sodium azide in THF as solvent. 1,2,3-triazoline derivatives (G1-G3) were synthesized by click reaction of unsaturated compounds like maleic anhydride, acrylamide and cinnamic acid with 2-azido gemcitabine in found sodium ascorbate and cuprous chloride as catalyst. The C.H.N. analysis of synthesized compounds was in agreement with the calculated percentage of elements. The FTIR spectra showed appearance of consumption of the azide of 2-azido gemcitabine and disappearance of the band at 2105 cm^{-1} due to the azide group. $^1\text{H-NMR}$ spectrum was considered good evidence for the formation of our compounds (Scheme 2).

Biological activity

All 1,2,3-triazoline compounds (G1-G3) were evaluated for their in-vitro α -glucosidase inhibition activity. All members of the series G1-G3 exhibited a potent α -glucosidase inhibition with IC_{50} values. All the tested compounds were found to be more active than the standard drug, acarbose ($\text{IC}_{50} = 824 \pm 1.73 \text{ mM}$). Compound G3 having a phenyl ring attached to 1,2,3-triazoline skeleton was found to be the least active member of the series with an IC_{50} value $345 \pm 4.5 \text{ mM}$. However, the activity increased when the 1,2,3-triazoline ring was binding with free amine group, as observed in compound G2 ($\text{IC}_{50} = 212.9 \pm 3.4 \text{ mM}$). Further increase in activity was observed for compound G1 ($\text{IC}_{50} = 144.8 \pm 1.74 \text{ mM}$) having the carbonyl groups in maleic anhydride ring. The results are summarized in Table 1.

Table 1 Results of α -glucosidase inhibition assay on compounds G1-G3

Compounds	Alpha-glucosidase inhibition, IC_{50} (μM)
G1	144 ± 1.74
G2	212 ± 3.4
G3	345 ± 4.5
Std. acarbose	824 ± 1.73

Conclusions

In this study, we synthesized and characterized a series of new 1,2,3-triazoline ring based on gemcitabine derivatives as α -glucosidase inhibitors. These compounds (G1-G3) were found to be more active than acarbose drug as in-vitro α -glucosidase inhibitors. The synthesized compounds could be candidates for antidiabetic drugs.

Conflict of Interests

The authors declare that no competing interest exists.

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