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RESEARCH ARTICLE

Synthesis and Design of New Gemcitabine Derivatives as *In Vitro* α-Glucosidase Inhibitors

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Abstract

A new series of gemcitabine derivatives containing imine structure have been prepared efficiently by reaction gemcitabine with some aromatic aldehydes. The structures of the imine derivatives were confirmed by many spectroscopic methods such as elemental analysis, FTIR and NMR spectroscopy. The α-glucosidase activity was evaluated of end compounds *in vitro*. All imine derivatives were showed α-glucosidase inhibition with IC_{50} values 223±1.24, 244±2.32, 94±1.01, 268±1.35, 387±2.12, 78±1.42, 145±1.32, 54±2.41 µM respectively, when compared with the control drug (IC_{50} = 784.35 ± 2.41 µM).Compound (g8) have higher therapeutic indices, representing possible promising roles. Overall result suggests that gemcitabine derivatives containing Schiff base structure (g1-g8) could be lead a new design in this study of novel α-glucosidase inhibitor.

Keywords: *Gemcitabine, Imine, Diabetes mellitus and α-Glucosidase Inhibitors.*

Introduction

Gemcitabine (GEM) is chemically known as [2′, 2′ difluorodeoxycytidine] [1]. In 1997 the first time has been using (GEM) a clinical treatment of pancreatic cancer. GEM is a type of chemotherapy to treat many types of cancer such as lung cancer, breast cancer and ovarian cancer. In addition to use as a prodrug [2-5]. International Diabetes Federation reports that, Diabetes mellitus is affects proximately 382 million people worldwide and is expected to rise in 2035 to beyond 592 million [6,7]. α-Glucosidase enzyme convert the polysaccharides to monosaccharide which leads to high blood sugar (hyperglycemia) in diabetic patients after eating [8,9].

A- Glycosidase inhibition was reported primarily to overcome the risk of hyperglycemia after ingestion in diabetics, Which is associated with many health disorders [10]. α-glucosidase inhibitors have developed many drugs such as Acarbose, Miglitol, 1-Dioxinogermesin, etc. but have adverse effects and low tolerance for patient [11,12]. In this study, successfully prepared a series of imine compounds derived from gemcitabine and tested in vitro the α-glucosidase inhibitory activity.

Experimental Information

All solvents used were redistilled. Silica-Gel (SG-40 Merck) is used in thin layer chromatography (T.L.C) with the solvents mentioned. Infrared spectra were recorded on Bruker ALPHA FT-IR spectrophotometer. CHNS elemental analysis was measurement by (204E) Perkin-Elmer Instrument. NMR spectrums were recorded by Bruker Instrument (^{1}H-NMR 300 MHz and ^{13}C-NMR 75 MHz).

General Method for Synthesis of imine Derivatives

A mixture 0.0122 mole of gemcitabine and 0.0122 mole of aromatic benzaldehyde (4-bromobenzaldehyde, 4-chlorobenzaldehyde,

4-nittrobenzaldehyde, 4-methylbenzaldehyde, *N, N*- dimethylbenzaldehyde, 4-hydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde and benzaldehyde) was dissolved in absolute ethanol (40 mL) then added some drops of glacial acetic acid, and refluxed the solution for (18-20) hrs. at 80 ^{o}C. The reaction progress was monitored by TLC. Then the product was extracted with 75 ml of diethyl ether, the organic phase was treated with 100 ml distill water. Subsequently, the diethyl ether phase was dried by adding Na_2SO_4 and filtered. The diethyl ether evaporated and the result residue was dried and purified by recrystallization from ethanol.

4-(4-(dimethylamino) benzylideneamino) Gemcitabine (g1)

It was prepared as a solid state, Chemical Formula: $C_{18}H_{20}F_2N_4O_4$; m.p. 188-190 ºC, yield 79% ; FTIR in cm^{-1}, 3453 for hydroxyl OH stretching group, 3113 for C-H aromatic stretching, 2966, 2843 for C-H aliphatic stretching**,** 1674 for C=O carbonyl group stretching, 1598, 1579, 1479 for C=N and C =C stretching**;** ^{1}HNMR (300 MHz,) δ 8.45(s,1HC=N imine), 7.66(d , J = 7.6 Hz, 1H-6, HC=N pyrimidine ring), 7.39-7.21 (m, Ar-H) and 6.42 (s, 1H-1), 5.89 (d, J = 7.6 Hz, for 1H-5, HC=N pyrimidine ring), 5.32 (brs, 1H-3), 4.58-4.44 (m, 3 H, 1H-4, 1H-a5, 1H-b5), 3.14 (s, 6H, N-(CH_3)$_2$; ^{13}C NMR at (75 MHz, DMSO-*d6*) show δ 166.21, 155.11, 144.45, 141.97,139.74, 128.35, 125.54,123.56, 93.46, 83.89, 77.38, 70.12, 62.61, 44.13; calculated: C=54.82; H= 5.11; F=9.63; N=14.21; O=16.2, Found: C=54.54; H=5.09; N=14.15.

4-(4 Methylbenzylideneamino) Gemcitabine (g2)

It was prepared as solid, Chemical Formula: $C_{17}H_{17}F_2N_3O_4$; m p 123-125 ºC, yield 74%; FTIR cm^{-1} 3412 for OH stretching group, at 3121 to C-H aromatic group, 2941, 2872 for C-H aliphatic stretching**,** 1673 for C=O carbonyl group stretching, 1583, 1537, 1473 for C=N and C =C groups; ^{1}HNMR in (DMSO-*d6*, 300 MHz) δ 8.48(s,1HC=N imine), 7.68(d , J = 7.6 Hz, 1H-6, HC=N pyrimidine ring), 7.35-7.23 (m, Ar-H), 6.39 (brs, 1H-1), 5.91 (d, J = 7.6 Hz, 1H-5, HC=N pyrimidine ring), 5.35 (m, 1H-3), 4.60-4.45 (m, 3 H, 1H-4, 1H-a5, 1H-b5), 2.41 (s, 3H, CH_3); ^{13}C-NMR at (75 MHZ, DMSO-*d6*) show δ 165.48, 156.01, 143.95, 142.17,133.71, 129.30, 126.65,125.87, 93.76, 82.97, 77.81, 70.21, 63.11, 22.31; calculated: C=55.89; H=4.69; F=10.40; N=11.50; O=17.52**,** Found: C=55.76; H=4.61; N=11.44.

4- (4 - Bromobenzylideneamino) Gemcitabine (g3)

It was prepared as sold, Chemical Formula: $C_{16}H_{14}BrF_2N_3O_4$; m p 144-146 ºC , yield 69% **;** FTIR cm^{-1} 3433 for OH hydroxyl group, 3141 for C-H aromatic group stretching , 2944, 2864 due to C-H groups**,** 1673 for C=O carbonyl group, 1587, 1547, 1470 for C=N and C =C groups; ^{1}H-NMR(DMSO-*d6*, 300 MHz) show δ 8.53(s,1HC=N imine), 7.67(d , J = 7.6 Hz, 1H-6, HC=N pyrimidine ring), 7.38-7.19 (m, Ar-H), 6.41 (s, 1H-1), 5.86 (d, J = 7.6 Hz, 1H-5, HC=N pyrimidine ring), 5.42 (m, 1H-3), 4.58-4.41 (m, 3 H, 1H-4, 1H-a5, 1H-b5); ^{13}C-NMR in (DMSO-*d6*, 75 MHz) δ 165.11, 155.42, 144.32, 132.65, 130.78, 129.74, 125.91, 94.87, 82.84, 78.21, 71.11, 62.88; calculate: C, 44.67; H, 3.28; and N, 9.77, Found: C, 44.48; H, 3.21; N, 9.72.

4-(4- hydroxybenzylideneamino) Gemcitabine (g 4)

It was prepared as solid, Chemical Formula: $C_{16}H_{15}F_2N_3O_5$; m p 165-167 ºC,, yield 73% **;** FTIR cm^{-1}, 3433 for hydroxyl OH stretching, 3122 for C-H aromatic group stretching, 2954, 2865 for C-H group stretching**,** 1577, 1535, 1480 for C=N and C =C stretching groups**;** ^{1}HNMR in (300 MHz, DMSO-*d6*) show δ 8.41 for (s,1HC=N imine), 8.04(s,1H, OH) 7.71(d , J = 7.6 Hz, 1H-6, HC=N pyrimidine ring), 7.32-7.22 (m, Ar-H), 6.34 (brs, 1H-1), 5.76 (d, J = 7.6 Hz for HC=N pyrimidine ring, 1H-5), 5.38 (m, 1H-3), 4.54-4.39 (m, 3 H, 1H-4, 1H-a5, 1H-b5); ^{13}C-NMR in (75 MHZ, DMSO-*d6*) δ 165.36, 155.68, 153.24, 144.74, 131.24, 129.87, 126.31,121.45, 94.42, 82.78, 77.59, 71.62, 62.69; calculate: C=52.32; H=4.12, N=11.44, Found: C=52.13; H=4.09 N=11.38.

4- (4- Hydroxyl - 3 methoxybenzylideneamino) Gemcitabine (g5)

It was prepared as solid, Chemical Formula: $C_{17}H_{17}F_2N_3O_6$; m p 192-194 ºC, yield 71% ; FTIR in cm^{-1}, 3428 for hydroxyl OH stretching, 3130 for C-H aromatic stretching, 2949, 2859 for C-H groups stretching, 1588, 1529, 1472 for C=N and C=C groups; ^{1}HNMR in (300 MHz, DMSO-*d6*) show δ 8.52 for (s, 1HC=N imine), 8.17(s,1H, OH) 7.73(d , J =

7.6 Hz, 1H-6, HC=N pyrimidine ring), 7.41-7.27 (m, Ar-H), 6.40 (brs, 1H-1), 5.77 (d, J = 7.6 Hz, 1H-5, HC=N pyrimidine ring), 5.42 (m, 1H-3), 4.56-4.41 (m, 3 H, 1H-4, 1H-a5, 1H-b5), 3.69 for (s,3H, O-CH_3); ^{13}C-NMR in (75 MHz, DMSO-*d6*) show δ 165.49, 155.79,147.12, 144.18,139.77, 129.68, 122.71,120.21, 118.89, 94.85, 82.46, 77.38, 70.99, 63.09, 54.57; calculated :C, 51.39; H, 4.31; N, 10.58, Found: C, 51.22; H, 4.28; N, 10.51.

4 - (4- Chlorobenzylideneamino) Gemcitabine (g6)

It was prepared as solid, Chemical Formula: $C_{16}H_{14}ClF_2N_3O_4$; m p 165-167 ºC, yield 70% **;** FTIR cm^{-1}, 3422 for OH group, 3120 for C-H aromatic group, and 2955, 2829 for C-H group**,** The 1588, 1535, 1487 for C=N and C=C stretching groups; ^{1}H-NMR in (300 MHz, DMSO-*d6*) show δ 8.61 (s,1HC=N imine), 7.70(d , J = 7.6 Hz, 1H-6, HC=N pyrimidine ring), 7.33-7.24 (m, Ar-H), 6.44 (brs, 1H-1), 5.76 (d, J = 7.6 Hz, 1H-5, HC=N pyrimidine ring), 5.41 (brs, 1H-3), 4.58-4.45 (m, 3 H, 1H-4, 1H-a5, 1H-b5); ^{13}C NMR(75 MHZ, DMSO-*d6*) δ 165.41, 155.68, 144.18, 135.71, 132.44, 130.65, 128.88, 94.65, 82.58, 77.74, 70.84, 62.87; calculated :C=49.82; H=3.66; N=10.89, Found: C=49.77; H=3.62; N=10.83.

4- (Benzylideneamino) Gemcitabine (g7)

It was prepared as solid, Chemical Formula: $C_{16}H_{15}F_2N_3O_4$; m p 188-190 ºC, yield 63% **;** FTIR cm^{-1}, 3447 for OH stretching, 3138 for C-H aromatic group, at 2944, 2854 for C-H stretching group**,** 1678 for C=O carbonyl group stretching, 1592, 1541, 1477 due to C=N and C =C stretching**;**

^{1}HNMR at (300 MHz, DMSO-*d6*) show δ 8.43 (s,1H for C=N imine), δ 7.71(d, 1H-6 for HC=N pyrimidine ring J=7.6 Hz), 7.35-7.23 (m, Ar-H), 6.41 (brs, 1H-1), 5.75 (d, J = 7.6 Hz, 1H-5, HC=N pyrimidine ring), 5.43 (m, 1H-3), 4.56-4.42 (m, 3 H, 1H-4, 1H-a5, 1H-b5); ^{13}C NMR(75 MHZ, DMSO-*d6*) δ 165.75, 155.52, 142.89, 132.44, 130.79, 129.24, 128.13, 93.78, 83.08, 77.24, 70.14, 61.98; calculated : C, 54.70; H, 4.30; N, 11.96, Found: C, 54.65; H, 4.28; N, 11.94.

4 - (4 -Nitrobenzylideneamino) Gemcitabine (g8)

It was prepared as sold, Chemical Formula: $C_{16}H_{14}F_2N_4O_6$; m p 111-113, yield 70% ºC**;** FTIR cm^{-1}, 3466 for OH stretching group, 3140 for C-H aromatic groups , 2968, 2856 for C-H stretching group**,** 1669 for C=O carbonyl group, 1589, 1539, 1473 for C=N and C=C stretching**;** ^{1}H-NMR at (300 MHz, DMSO-*d6*) show δ 8.77(s,1HC=N imine), 7.73 (d , J = 7.6 Hz, 1H-6, HC=N pyrimidine ring), 7.41-7.24 (m, Ar-H), 6.44 (s, 1H-1), 5.73 (d, J = 7.6 Hz, 1H-5, HC=N pyrimidine ring), 5.40 (brs, 1H-3), 4.51-4.35 (m, 3 H, 1H-4, 1H-a5, 1H-b5);

^{13}C-NMR at (75MHz, DMSO-*d6*) show δ 166.15, 155.87, 148.54, 145.76, 137.87, 128.38, 126.42, 94.08, 84.16, 76.84, 714.64, 61.68; calculated: C= 48.49; H=3.56; N=14.14, Found: C=48.39; H=3.51; N=14.11.

Glucosidase Inhibition Analysis [13]

All imine derivatives (g1-g8) where tested α-Glucosidase *in vitro* enzyme inhibitory activity. Acarbose drug used as stander drug and (p-nitrophenyl glucopyranoside) as substrate. Enzyme solution was prepared in buffer saline solution of potassium phosphate (pH 7.3, 0.02M), end product samples (g1-g8) were prepared in (10% final concentration). 120 µL of potassium phosphate buffer, 30 µL of end product was added in several concentrations, and was added (25 µL 0.2 Unit/ml) of α-Glucosidase enzyme solution and incubated at 37 ^{o}C for 10 min.

And after that, (25 µL, 0.5 µM) of substrate (p-nitrophenyl glucopyranoside) was added to the mixture and incubated also at 37 ^{o}C for 30 min. Finally, recorded the absorbance at 405 nm by using spectrophotometer. 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 calculated by using this formula:

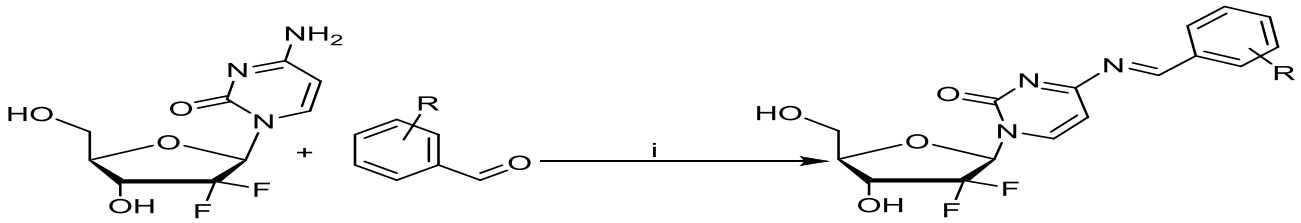
% Inhibition= [Abs control – Abs sample / Abs control] * 100%

Results and Discussion

Synthesis

The gall of this paper is to produce new imine compounds of gemcitabine Scheme 1. The target compounds (g1-g8) have been prepared by reaction gemcitabine and aromatic benzaldehyde (4- bromobenzaldehyde, 4-chlorobenzaldehyde, 4- nittrobenzaldehyde, 4- methylbenzaldehyde, *N, N*- dimethyl benzaldehyde, 4-hydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde and

benzaldehyde) in absolute ethanol and presence some drops of glacial acetic acid.



R=
4-N,N-dimethyl
4-Br
4-OH
3-methoxy-4OH
4-Cl
4-methyl
4-H
4-NO_2

i=
glacial acetic acid
Abs.Ethanol
80 ^{o}C
18-20 hours

Scheme 1: Synthetic protocol for Schiff base derivatives of gemcitabine

The chemical structures of all the end products were identified by some spectroscopic methods including ^{1}HNMR, ^{13}CNMR, FTIR, and elemental analysis. The ^{1}HNMR spectrum of compounds **g1-g8** showed the absence signal for the amines proton at(δ 2.4 ppm) in gemcitabine and showed that there is a new signal in range (a singlet at δ 8.41-8.77 ppm) for the protons of imine group N=C**H**.

The ^{13}CNMR spectrum of imine compounds showed that there is appearance a new signal of the carbon imine group N=**C**H at 142-145 ppm. On the other hand the FT-IR spectra showed the appearance of the stretching vibration between1598-1577cm^{-1} imine group in compounds **g1-g8** and disappearance of two absorption band of the asymmetric and symmetric stretching vibration of NH_2 group in range3380-3249cm^{-1}.

Biological Activity

The second gall of this work was evaluated α-glucosidase *in-vitro* inhibition of imine compounds. All Schiff base compounds **g1-g8** shows active α-glucosidase inhibition as in IC_{50} values **(Fig.1.)** 110 ± 2.15, 197 ± 3.11, 38 ± 0.84, 64 ± 1.78, 119 ± 3.55, 204 ± 2.08, 32 ± 1.42, 81 ± 2.23 respectively.

All compounds found more active than the acarbose standard drug (IC50 = 824 ± 1.73 - μM). Compounds **g8**, g6 and g3 having a phenyl ring was substituted with nitro, chloro and bromo groups at para position were show the highest active of the other series [14,15] at IC_{50} value 32 ± 1.42, 38 ± 0.84 μM respectively. While the inhibition activity decreased when the phenyl ring attach with donating groups so the compound g5 was less effective among all prepared derivatives.

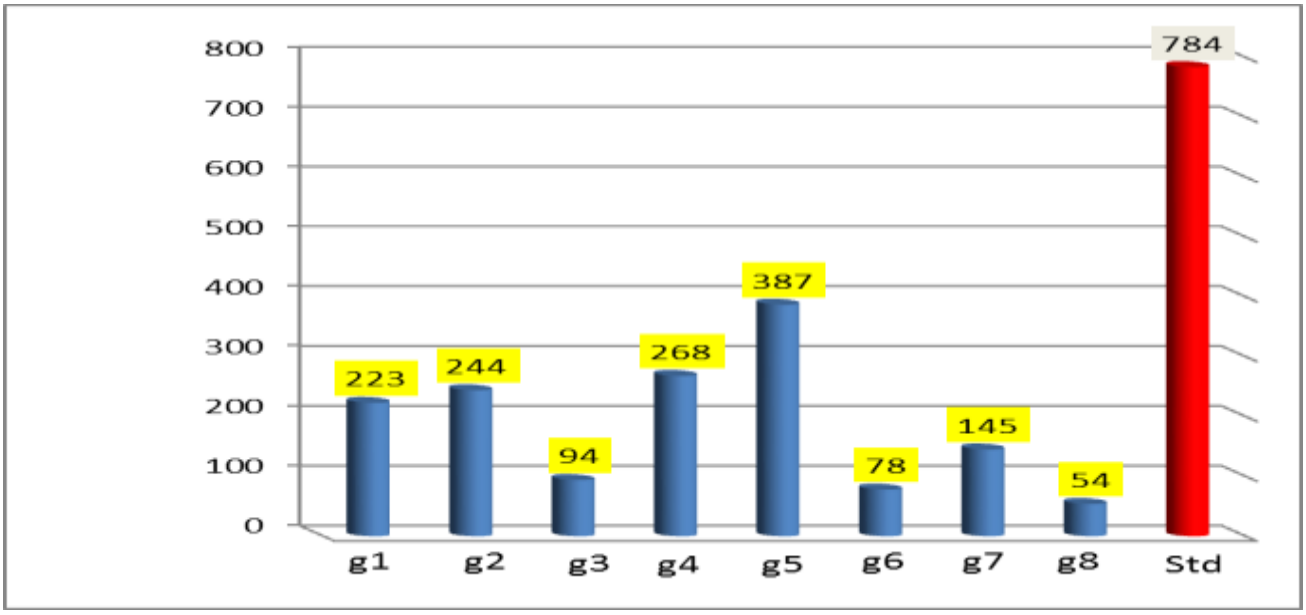


Fig.1: IC_{50} α-glucosidase inhibition compounds of g1-g8

Conclusion

In this paper, prepared and identified a series of new gemcitabine derivatives as α-glucosidase inhibitors. The target compounds g1-g8 has more active than acarbose drug, *in vitro* α-glucosidase inhibitors. These results indicate that inhibitory activity of the imine derivatives increase when structure contains substituted aryl with a withdrawing group. So these compounds may be used as potential candidates in search for anti-diabetic drugs.

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