doi.org/10.36721/PJPS.2021.34.5.SUP.1909-1915.1

Synthesis and α-glucosidase inhibition studies of norfloxacin-acetanilide hybrids

Kamran Walayat[1], Matloob Ahmad[1]*, Usman Ali Ashfaq[2], Zulfiqar Ali Khan[1] and Sadia Sultan[3,4]*

[1]Department of Chemistry, Government College University, Faisalabad, Pakistan
[2]Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan
[3]Faculty of Pharmacy, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia
[4]Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia

Abstract: α-Glucosidase inhibitors occupy a prominent position among the various treatments of type-2 diabetes mellitus (DM2). In this study, a series of new norfloxacin-acetanilide hybrid molecules were synthesized and screened for α-glucosidase inhibition activity. The synthetic methodology involves the synthesis of a series of α-bromoacetanilides by condensing bromoacetyl bromide with various substituted anilines. These α-bromoacetanilides were coupled with norfloxacin in DMF to get the titled hybrids. The structure elucidation of synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and LC-MS. Finally, the compounds were screened for their α-glucosidase inhibition activity using acarbose as a reference drug (IC_{50} =58 µM). Among the tested compounds, 3i and 3j displayed potent α-glucosidase inhibition activity with IC_{50} values of 7.81±0.038 and 5.55±0.012 µM respectively. In-addition, 3m, 3f and 3k were demonstrated moderate alpha-glucosidase inhibition activities with IC_{50} values of 52.905±0.041, 23.79± 0.087 and 23.06±0.026 µM respectively. The structure-activity relationship was established with the help of molecular docking by using Molecular Operating Environment software (MOE 2014).

Keywords: Norfloxacin derivatives, α-glucosidase inhibition activity, anti-diabetic studies, molecular docking.

INTRODUCTION

Diabetes mellitus (DM) is one of the major health problems across the globe and its prevalence rate is growing rapidly leading towards higher morbidity and mortality rates (Jimenez-Montero, 2019). According to the estimates of the International Diabetes Federation (IDF), the number of people suffering with the DM in year 2017 was 451 million and its occurrence rate is projected to 693 million by the year 2045 (Cho *et al*., 2018). Acarbose, miglitol and voglibose are extensively used anti-diabetic drugs to treat postprandial hyperglycemia (Kulkarni *et al*., 2017). Oral antidiabetic drugs have been categorized as biguanides, meglitinides, sulfonylureas, dipeptidyl peptidase IV inhibitors, thiazolidinediones and α-glucosidase inhibitors. These drugs include repaglinide, pioglitazone, sitagliptin, metformin, glimepiride and acarbose etc. (Qian *et al.,* 2018).

α-Glucosidase performs its function by catalyzing the hydrolysis of complex carbohydrates into simple carbohydrates enabling their easy absorption. Thus, α-glucosidase inhibitors find their application in delaying the absorption of sugar after meals. These compounds are useful for the treatment of type 2 diabetes mellitus. Currently, only three inhibitors are in clinical use, such as acarbose, miglitol and voglibose (Dash *et al*., 2018). Fluoroquinolones are a group of antibiotics widely used to treat severe bacterial infections. These drugs include ciprofloxacin, enoxacin, levofloxacin, gatifloxacin and norfloxacin etc. These drugs are also acting as scaffolds for the discovery of new biologically active molecules. Ciprofloxacin derivatives have been reported as anti-HIV (Sriram *et al*., 2007), anti-tuberculosis (Suresh *et al*., 2014), antibacterial and antifungal (Zhang *et al*., 2018), anticancer (Teyhoo *et al*., 2020; Akhtar *et al.,* 2021) and to treat complicated urinary tract infactions (Sabo *et al*., 2015) etc. In-addition, various derivatives of norfloxacin have been reported as antimicrobial (Oniga *et al*., 2018; Walayat *et al.,* 2020) and anticancer (Yadav and Talwar, 2019) etc. Keeping in view the versatile bioactivity profile of fluoroquinolones, we synthesized norfloxacin hybrids with acetanilides and studied their hypoglycemic activity through α-glucosidase inhibition activity.

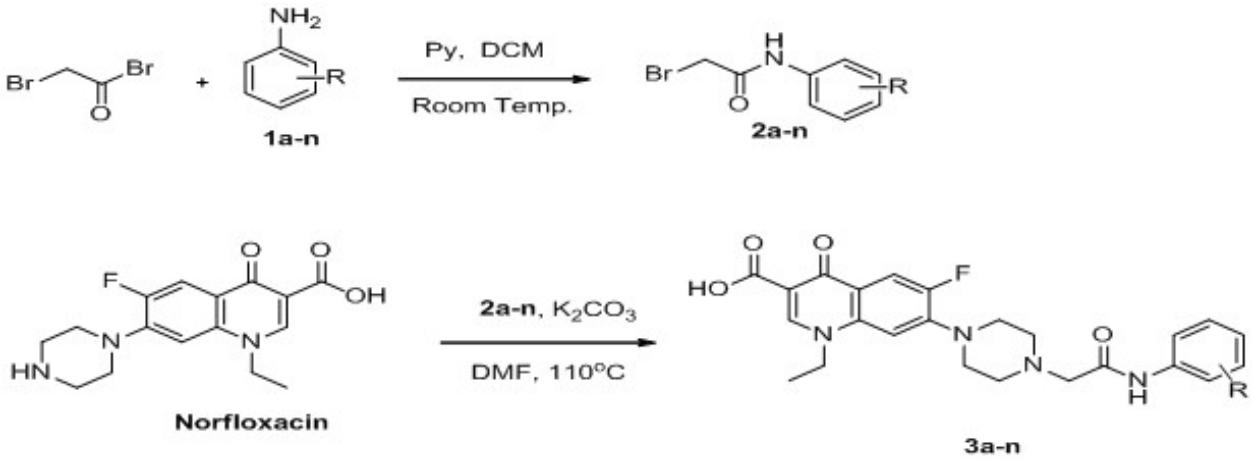
MATERIALS AND METHODS

General experimental part

All the chemicals were purchased from Sigma Aldrich. ^{1}H NMR spectra were recorded at Bruker instrument at 600 MHz. Mass spectra of synthesized compounds were acquired on LC-MS instrument. The precoated silica gel aluminum plates (Kieselgel 60, F254, Merck, Germany) were used to perform thin layer chromatography (TLC).

α-Bromoacetanilides 2a-n were prepared according to

**Corresponding author:* e-mail: matloob.ahmad@gcuf.edu.pk; drsadia@uitm.edu.my



Scheme 1: Synthetic layout for the preparation of norfloxacin-acetanilide hybrids 3a-n.

reported methodology (Zhang *et al.,* 2018; Adhikary and Lee, 2011).

General procedure for the synthesis of norfloxacin-acetanilide hybrid derivatives 3a-n

Equimolar quantities of norfloxacin (1.57 mmol), substituted acetanilides (1.57 mmol) and K_2CO_3 (3.14 mmol) were mixed in dimethylformamide (DMF) to synthesize novel norfloxacin-acetanilides (3a-n). The mixture was kept on heating at 110-120^oC until the completion of the reaction (as indicated by the TLC). On completion of chemical reaction, the mixture was cooled to room temperature and was poured on crushed ice. The precipitates of the synthesized compounds were obtained which were purified via silica-gel column chromatography using n-hexane/chloroform mixture as eluent yielding 100% pure compounds. Afterwards, the collected samples were treated to remove solvents by rotary evaporation. The spectral data for the structure elucidation is provided in table 1.

Docking procedure

The molecular docking studies was performed by using MOE 2014 (Inc., 2016) according to the reported methodology (Saddique et al., 2019). Active sites of receptor molecule containing Asp(A203), Asp(A542), Asp(A327), His(A600), Arg(A526) were selected. The 2D structure of synthetic compounds was drawn by Chem-Draw. The optimization of ligand was done through adding partial charges using Protonate 3Dalgorithm implemented in MOE (Inc., 2016) and MMFF94X force field was applied for energy minimization. The optimized ligands were then added into the MOE ligand database for docking study. After docking, the best and top conformations were determined on the basis of S-score and RMSD value.

α-Glucosidase inhibition assay

α-Glucosidase inhibition assays of the synthesized derivatives of norfloxacin was conducted according to previously reported protocols with slight modifications (Şöhretoğlu *et al*., 2017; Mohammadi-Khanaposhtani *et al.,* 2018) as well as in our work (Taj *et al*., 2019). The in-vitro results are described in table 2.

STATISTICAL ANALYSIS

All experiments were conducted in replicate. *Mean of experiments are presented, n = 3 and $p < 0.05$ were considered statistically significant (Ms. Excel 2010).

RESULTS

Chemistry

Protocols for the synthesis of target compounds are illustrated in scheme 1. All the synthesized compounds are characterized using various spectroscopic techniques like LC-MS, ^1H NMR and ^{13}C NMR as shown in table 1. Analytical methods like thin layer chromatography (TLC) and column chromatography are also employed to confirm the completion and purification of the targeted compounds respectively.

In vitro screening

All the synthetic norfloxacin derivatives were screened to evaluate their *in vitro* α-glucosidase inhibitory activity with reference to acarbose as a standard inhibitor (table 2). Following formula is used to calculate the IC_{50} values of the compounds.

$$IC_{50} = X_1 + M (50 - Y_1)$$

Where $M = (X_2-X_1) / (Y_2-Y_1)$; X_2= Just above 50 (Dose Response), Y_2 = Just below 50 (Dose response), X_1= Just above 50% inhibition, Y_1= Just below 50% inhibition. Docking Scores, rmsd and interacting residues for the titled norfloxacin derivatives have been presented in table 3.

Table 1: Spectral data of the compounds 3a-n.

Compound	Yield (%)	Characterization Data
Norfloxacin	-	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.41 (t, *J*=7.09 Hz, 3H, CH_3), 2.88 (t, *J*=4.64 Hz, 4H, Pip), 3.23 (t, *J*=4.61 Hz, 4H, Pip), 4.56 (q, *J*=14.21, 7.09 Hz, 2H, NCH_2CH$_3$), 7.13 (d, *J*= 7.18 Hz, 1H, Ar-*H*), 7.88 (d, *J*=13.36 Hz, 1H, Ar-*H*), 8.91 (s, 1H, Ar-*H*); LC-MS (m/z): calcd: 318[M-H]$^-$, 320[M+H]$^+$, 342[M+Na]$^+$; found: 318[M-H]$^-$, 320[M+H]$^+$, 342 [M+Na]$^+$.
3a	93%	^{1}H NMR (CDCl$_3$, 600 MHz) δ: 1.63 (t, *J*=8.67 Hz, 3H, CH_3), 2.34 (s, 3H, CH_3-Ar), 2.97 (br-s, 4H, Pip), 3.34 (s, 2H, NCH_2CO), 3.45 (br-s, 4H, Pip), 4.36 (q, *J*=17.43, 8.7 Hz, 2H, NCH_2CH$_3$), 6.88 (d, *J*=8.16 Hz, 1H, Ar-*H*), 7.1 (t, *J*=8.88 Hz, 2H, Ar-*H*), 7.21-7.27 (m, 1H, Ar-*H*), 8.1-8.13 (m, 2H, Ar-*H*), 8.7 (s, 1H, Ar-*H*), 9.16 (br-s, 1H, CON*H*), 15.04 (s, 1H, COO*H*); ^{13}C NMR (CDCl$_3$, 150 MHz) δ: 14.5, 17.9, 49.71, 49.89, 50.15, 53.32 (2C), 62.12, 103.89, 108.55, 112.99, 113.18, 121.03, 124.69, 126.91, 127.12, 130.41, 135.57, 137.08, 145.58, 147.19, 152.46, 167.07, 167.41, 176.98. LC-MS (m/z): calcd: 465 [M-H]$^-$, 467 [M+H]$^+$, 489[M+Na]$^+$, 505[M+K]$^+$; found: 465[M-H]$^-$, 467[M+H]$^+$, 489[M+Na]$^+$, 505[M+K]$^+$.
3b	89%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.42 (t, *J*=5.4 Hz, 3H, CH_3), 2.28 (s, 3H, CH_3-Ar), 2.73-2.75 (m, 4H, Pip), 3.22 (s, 2H, NCH_2CO), 3.39-3.41 (m, 4H, Pip), 4.57 (q, *J*=17.43, 7.14 Hz, 2H, NCH_2CH$_3$), 6.89 (d, *J*=7.56 Hz, 1H, Ar-*H*), 7.19 (q, *J*=15.18, 7.54 Hz, 2H, Ar-*H*), 7.43-7.45 (m, 2H, Ar-*H*), 7.89-7.94 (m, 1H, Ar-*H*), 8.92 (s, 1H, Ar-*H*), 9.72 (s, 1H, CON*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.27, 21.07, 49.11, 49.32, 49.35, 52.25 (2C), 61.35, 105.66, 106.96, 116.66, 119.07, 119.12, 120.01, 124.21, 128.5, 137.18, 137.91, 137.3, 145.41, 145.48, 148.33, 166.21, 168.11, 176.1. LC-MS (m/z): calcd: 465 [M-H]$^-$, 467 [M+H]$^+$, 505 [M+K]$^+$; found: 465 [M-H]$^-$, 467 [M+H]$^+$, 505 [M+K]$^+$.
3c	89%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.43 (t, *J*=7.11 Hz, 3H, CH_3), 2.27 (s, 3H, CH_3-Ar), 2.55 (s, 4H, Pip), 3.22 (s, 2H, NCH_2CO), 4.24 (s, 4H, Pip), 4.61 (q, *J*=14.16, 7.06 Hz, 2H, NCH_2CH$_3$), 7.16 (d, *J* = 8.22 Hz, 3H, Ar-*H*), 7.28 (d, *J*=7.14 Hz, 1H, Ar-*H*), 7.52 (d, *J* = 8.28 Hz, 2H, Ar-*H*), 7.97 (d, *J*=12.96 Hz, 1H, Ar-*H*), 8.95 (s, 1H, CON*H*), 10.79 (br-s, 1H, COO*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.4, 20.41, 34.14, 46.4, 46.43, 46.46, 49.21, 51.43, 106.47, 107.11, 111.33, 111.48, 119.55, 119.89, 119.94, 129.26, 133.3, 135.32, 137.11, 148.59, 151.85, 153.51, 166.1, 176.13, 176.15. LC-MS (m/z): calcd: 465 [M-H]$^-$, 467 [M+H]$^+$, 505 [M+K]$^+$; found: 465 [M-H]$^-$, 467 [M+H]$^+$, 505 [M+K]$^+$.
3d	91%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.43 (t, *J*=7.14 Hz, 3H, CH_3), 2.73 (s, 4H, Pip), 2.89 (s, 2H, NCH_2CO), 3.85 (s, 3H, OCH_3), 4.24 (br-s, 4H, Pip), 4.6 (q, *J*=13.95, 6.94 Hz, 2H, NCH_2CH$_3$), 6.97 (t , *J*=7.59 Hz, 1H, Ar-*H*), 7.1 (d , *J*=8.04 Hz, 1H, Ar-*H*), 7.17 (t , *J*=7.8 Hz, 1H, Ar-H), 7.27 (d , *J*=7.08 Hz, 1H, Ar-*H*), 7.91 (d , *J*=7.74 Hz, 1H, Ar-*H*), 7.97 (d , *J*= 13.02 Hz, 1H, Ar-*H*), 8.95 (s, 1H, Ar-*H*), 9.99 (s, 1H, CON*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.37, 46.47, 46.52, 46.54, 49.21, 51.59, 55.71 (2C), 106.4, 107.11, 111.35, 111.45, 111.5, 119.89, 120.33, 125.63, 125.78, 137.1, 144.06, 148.58, 149.97, 151.86, 153.51, 166.13, 176.14. LC-MS (m/z): calcd: 481 [M-H]$^-$, 483 [M+H]$^+$, 521[M+K]$^+$; found: 481[M-H]$^-$, 483[M+H]$^+$, 521[M+K]$^+$.
3e	94%	^{1}H NMR (CDCl$_3$, 600 MHz) δ: 1.61 (t, *J*=7.29 Hz, 3H, CH_3), 2.88 (t, *J*=4.56 Hz, 4H, Pip), 3.26 (s, 2H, NCH_2CO), 3.42 (t, *J*=4.56 Hz, 4H, Pip), 3.81 (s, 3H, OCH_3), 4.34 (q, *J*=14.52, 7.26 Hz, 2H, NCH_2CH$_3$), 6.86-6.89 (m, 3H, Ar-*H*), 7.48 (dd, *J*=6.9, 1.98 Hz, 2H, Ar-*H*), 8.09 (d, *J*=12.9 Hz, 1H, Ar-*H*), 8.68 (s, 1H, Ar-*H*), 8.88 (br-s, 1H, CON*H*), 15.05 (s, 1H, COO*H*); ^{13}C NMR (CDCl$_3$, 150 MHz) δ: 14.50, 49.72, 49.98, 53.15 (2C), 55.52 (2C), 61.88, 103.90, 108.52, 114.25 (2C), 120.92, 120.98, 121.27 (2C), 130.54, 137.07, 145.68, 145.75, 147.21, 156.53, 167.12, 167.34, 177.01. LC-MS (m/z): calcd: 481 [M-H]$^-$, 483 [M+H]$^+$, 505 [M+Na]$^+$, 521 [M+K]$^+$; found: 481 [M-H]$^-$, 483 [M+H]$^+$, 505 [M+Na]$^+$, 521 [M+K]$^+$.
3f	86%	^{1}H NMR (CDCl$_3$, 600 MHz) δ: 1.55 (t, *J*=6.1 Hz, 3H, CH_3), 2.85 (t, *J*=4.66 Hz, 4H, Pip), 3.25 (s, 2H, NCH_2CO), 3.38 (t, *J*=4.39 Hz, 4H, Pip), 4.28 (q, *J*=14.55, 7.27 Hz, 2H, NCH_2CH$_3$), 6.8 (d, *J*=6.81 Hz, 1H, Ar-*H*), 7.0 (td, *J*=7.84, 1.53 Hz, 1H, Ar-*H*), 7.23-7.26 (m, 1H, Ar-*H*), 7.32 (dd, *J*=8.03, 1.39 Hz, 1H, Ar-*H*), 8.03 (d, *J*=12.94 Hz, 1H, Ar-*H*), 8.42 (dd, *J*=8.27, 1.39 Hz, 1H, Ar-*H*), 8.62 (s, 1H, Ar-*H*), 9.81 (s, 1H, CON*H*), 14.97 (s, 1H, COO*H*); ^{13}C NMR (CDCl$_3$, 150 MHz) δ: 14.5, 49.72 (2C), 50.11, 53.08 (2C), 62.04, 103.91, 108.57, 113.0, 113.15, 121.02, 122.59, 124.74, 127.99 (2C), 129.05 (2C), 134.34, 137.11, 147.2, 167.16, 167.87, 177.06. LC-MS (m/z): calcd: 485 [M-H]$^-$, 487 [M+H]$^+$; found: 485 [M-H]$^-$, 487 [M+H]$^+$.
3g	82%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.42 (t, *J*=7.05 Hz, 3H, CH_3), 2.74 (br-s, 4H, Pip), 3.25 (s, 2H, NCH_2CO), 3.39 (br-s, 4H, Pip), 4.56 (q, *J*=14.16, 7.06 Hz, 2H, NCH_2CH$_3$), 7.13 (td, *J*= 20.76, 6.66 Hz, 2H, Ar-*H*), 7.34 (t, *J*=8.1 Hz, 1H, Ar-*H*), 7.54 (d, *J*=8.04 Hz, 1H, Ar-*H*), 7.84 (s, 1H, Ar-*H*), 7.89 (d, *J*=13.32 Hz, 2H, Ar-*H*), 8.9 (s, 1H, CON*H*), 10.01 (s, 1H, COO*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.3, 49.12, 49.23, 49.26, 52.21 (2C), 61.26, 105.62, 106.94, 111.05, 111.2, 117.92, 119.04, 123.2, 130.35, 132.98, 137.16, 139.86, 145.4, 145.46, 148.29, 166.23, 168.66, 176.07. LC-MS (m/z): calcd: 485 [M-H]$^-$, 487 [M+H]$^+$, 509 [M+Na]$^+$, 525 [M+K]$^+$; found: 485 [M-H]$^-$, 487 [M+H]$^+$, 509 [M+Na]$^+$, 525 [M+K]$^+$.
3h	93%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.43 (t, *J*=7.14 Hz, 3H, CH_3), 2.52-2.55 (m, 4H, Pip), 3.82 (s, 2H, NCH_2CO), 4.27 (s, 4H, Pip), 4.6 (q, *J*=14.16, 7.06 Hz, 2H, NCH_2CH$_3$), 7.26 (d, *J*=7.2 Hz, 1H, Ar-*H*), 7.42 (dd, *J* = 7.02, 2.76 Hz, 2H, Ar-*H*), 7.66 (d, *J*=8.88 Hz, 2H, Ar-*H*), 7.95 (d, *J* = 13.02 Hz, 1H, Ar-*H*), 8.93 (s, 1H, Ar-*H*), 10.98 (br-s, 1H, CON*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.32, 46.34, 46.36, 49.2, 51.5 (2C), 56.81, 106.41, 107.09, 111.32, 119.94, 121.14 (2C), 127.9, 128.78 (2C), 136.73, 137.07, 143.89, 143.96, 148.54, 163.06, 166.15, 176.09. LC-MS (m/z): calcd: 485 [M-H]$^-$, 487 [M+H]$^+$; found: 485 [M-H]$^-$, 487 [M+H]$^+$.

Continue…

3i	85%	^{1}H NMR ($CDCl_3$, 600 MHz) δ: 1.54 (t, *J*=6.57 Hz, 3H, C*H*$_3$), 2.86 (t, *J*=4.73 Hz, 4H, Pip), 3.24 (s, 2H, NC*H*$_2$CO), 3.4 (t, *J*=4.5 Hz, 4H, Pip), 4.27 (q, *J*=14.55, 7.27 Hz, 2H, NC*H*$_2$CH$_3$), 6.79 (d, *J*=6.83 Hz, 1H, Ar-*H*), 6.94 (td, *J* = 7.94, 1.57 Hz, 1H, Ar-*H*), 7.28 (t, *J*=8.43 Hz, 1H, Ar-*H*), 7.48 (dd, *J*=8.03, 1.36 Hz, 1H, Ar-*H*), 8.01 (d, *J* = 12.97 Hz, 1H, Ar-*H*), 8.42 (dd, *J* = 8.26, 1.48 Hz, 1H, Ar-*H*), 8.61 (s, 1H, Ar-*H*), 9.8 (s, 1H, CON*H*), 14.98 (s, 1H, COO*H*); ^{13}C NMR ($CDCl_3$, 150 MHz) δ: 14.49, 49.72, 50.07, 50.1, 53.1 (2C), 62.08, 103.85, 108.53, 112.97, 113.02, 121.23, 125.24, 128.63, 132.29, 135.5, 137.12, 145.67, 147.17, 152.64, 154.31, 167.15, 167.99, 177.03. LC-MS (m/z): calcd: 529 $[M-H]^-$, 531 $[M-H]^-$, 531 $[M+H]^+$, 533 $[M+H]^+$, 555 $[M+Na]^+$, 569 $[M+K]^+$, 571 $[M+K]^+$; found: 529 $[M-H]^-$, 531 $[M-H]^-$, 531 $[M+H]^+$, 533 $[M+H]^+$, 555 $[M+Na]^+$, 569 $[M+K]^+$, 571 $[M+K]^+$.
3j	89%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.43 (t, *J*=7.14 Hz, 3H, C*H*$_3$), 2.52 (s, 4H, Pip), 3.83 (s, 2H, NC*H*$_2$CO), 4.28 (s, 4H, Pip), 4.6 (q, *J*=14.55, 4.72 Hz, 2H, NC*H*$_2$CH$_3$), 7.27 (d, *J*=7.2 Hz, 2H, Ar-*H*), 7.33-7.35 (m, 2H, Ar-*H*), 7.55 (dt, *J*=7.02, 1.92 Hz, 1H, Ar-*H*), 7.95-7.97 (m, 2H, Ar-*H*), 8.95 (s, 1H, CON*H*), 11.05 (br-s, 1H, COO*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.41, 34.22, 46.4, 49.18, 51.49 (2C), 56.82, 106.43, 107.12, 111.33, 118.34, 119.95, 121.91, 126.88, 130.97, 137.08, 139.36, 143.97, 148.58, 151.83, 153.48, 163.33, 166.08, 176.11. LC-MS (m/z): calcd: 529 $[M-H]^-$, 531 $[M-H]^-$, 531 $[M+H]^+$, 533 $[M+H]^+$, 571 $[M+K]^+$; found: 529 $[M-H]^-$, 531 $[M-H]^-$, 531 $[M+H]^+$, 533 $[M+H]^+$, 571 $[M+K]^+$.
3k	95%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.43 (t, *J*=7.14 Hz, 3H, C*H*$_3$), 2.52 (s, 4H, Pip), 3.83 (s, 2H, NC*H*$_2$CO), 4.25 (s, 4H, Pip), 4.6 (q, *J*=13.89, 6.9 Hz, 2H, NC*H*$_2$CH$_3$), 7.26 (d, *J*=7.14 Hz, 1H, Ar-*H*), 7.54-7.63 (m, 5H, Ar-*H*), 7.96 (d, *J*=12.96 Hz, 1H, Ar-*H*), 8.94 (s, 1H, CON*H*), 10.92 (br-s, 1H, COO*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.32, 46.36, 46.38, 49.2, 51.53 (2C), 56.89, 106.42, 107.11, 115.97, 119.91, 119.96, 121.51 (2C), 131.78 (2C), 137.1, 143.91, 143.98, 148.58, 151.84, 163.11, 166.11, 176.11. LC-MS (m/z): calcd: 529 $[M-H]^-$, 531 $[M-H]^-$, 531 $[M+H]^+$, 533 $[M+H]^+$, 553 $[M+Na]^+$; found: 529 $[M-H]^-$, 531 $[M-H]^-$, 531 $[M+H]^+$, 533 $[M+H]^+$, 553 $[M+Na]^+$.
3l	93%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.43 (t, *J*=7.17 Hz, 3H, C*H*$_3$), 2.52 (s, 4H, Pip), 3.33 (s, 2H, NC*H*$_2$CO), 4.32 (s, 4H, Pip), 4.58 (q, *J*=13.89, 4.76 Hz, 2H, NC*H*$_2$CH$_3$), 7.26 (d, *J*=7.2 Hz, 1H, Ar-*H*), 7.67 (t, *J* = 8.19 Hz, 1H, Ar-*H*), 7.92-7.95 (m, 3H, Ar-*H*), 7.98-8.0 (m, 1H, Ar-*H*), 8.66 (t, *J*=2.1 Hz, 1H, Ar-*H*), 8.92 (s, 1H, CON*H*), 11.34 (s, 1H, COO*H*); LC-MS (m/z): calcd: 496 $[M-H]^-$, 498 $[M+H]^+$, 536 $[M+K]^+$; found: 496 $[M-H]^-$, 498 $[M+H]^+$, 536 $[M+K]^+$.
3m	93%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.43 (t, *J*=7.17 Hz, 3H, C*H*$_3$), 2.52 (s, 4H, Pip), 3.33 (s, 2H, NC*H*$_2$CO), 4.32 (s, 4H, Pip), 4.58 (q, *J* = 13.89, 4.76 Hz, 2H, NC*H*$_2$CH$_3$), 7.26 (d, *J*=7.2 Hz, 1H, Ar-*H*), 7.67 (t, *J*= 8.19 Hz, 1H, Ar-*H*), 7.92-7.95 (m, 3H, Ar-*H*), 7.98-8.0 (m, 1H, Ar-*H*), 8.66 (t, *J* = 2.1 Hz, 1H, Ar-*H*), 8.92 (s, 1H, CON*H*), 11.34 (s, 1H, COO*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.31, 42.6, 46.33, 46.37, 49.22, 51.58, 56.81, 106.38, 107.07, 111.31, 111.46, 113.72, 118.81, 125.56, 130.46, 137.06, 138.84, 143.89, 147.91, 148.5, 151.83, 163.73, 166.14, 176.08. LC-MS (m/z): calcd: 496 $[M-H]^-$, 498 $[M+H]^+$, 520 $[M+Na]^+$, 536 $[M+K]^+$; found: 496 $[M-H]^-$, 498 $[M+H]^+$, 520 $[M+Na]^+$, 536 $[M+K]^+$.
3n	91%	^{1}H NMR (DMSO-d_6, 600 MHz) δ: 1.43 (t, *J*=7.11 Hz, 3H, C*H*$_3$), 2.52 (s, 4H, Pip), 3.83 (s, 2H, NC*H*$_2$CO), 4.23 (s, 4H, Pip), 4.61 (q, *J*=14.02, 6.97 Hz, 2H, NC*H*$_2$CH$_3$), 7.27 (d, *J*=7.12 Hz, 1H, Ar-*H*), 7.89 (d, *J*= 9.17 Hz, 2H, Ar-*H*), 7.97 (d, *J*=13.0 Hz, 1H, Ar-*H*), 8.28 (d, *J*=9.17 Hz, 3H, Ar-*H*), 8.95 (s, 1H, CON*H*); ^{13}C NMR (DMSO-d_6, 150 MHz) δ: 14.39, 46.73, 49.18 (2C), 51.65 (2C), 56.81, 106.37, 107.12, 111.33, 111.49, 119.38 (2C), 125.04 (2C), 137.11, 142.87, 143.97, 148.61 (2C), 151.87, 153.53, 166.11, 176.16. LC-MS (m/z): calcd: 496 $[M-H]^-$, 498 $[M+H]^+$, 536 $[M+K]^+$; found: 496 $[M-H]^-$, 498 $[M+H]^+$, 536 $[M+K]^+$.

Table 2: Structural parameters and α-glucosidase inhibition activity of norfloxacin based acetanilides.

Sr. No.	Compound	R	% Inhibition	IC_{50} Value (μM)
1.	Norfloxacin		43.21	7.95±0.018
2.	3a	2-Methyl	35.52	N.A
3.	3b	3-Methyl	ND	N.A
4.	3c	4-Methyl	57.21	106.96±0.11
5.	3d	2-Methoxy	69.45	62.51±0.03
6.	3e	4-Methoxy	39.72	128.75±0.104
7.	3f	2-Chloro	53.30	23.79±0.087
8.	3g	3-Chloro	4.52	N.A
9.	3h	4-Chloro	ND	N.A
10.	3i	2-Bromo	33.93	7.81±0.038
11.	3j	3-Bromo	44.27	5.55±0.012
12.	3k	4-Bromo	47.67	23.06±0.026
13.	3l	2-Nitro	44.66	62.5±0.106
14.	3m	3-Nitro	73.41	52.905±0.041
15.	3n	4-Nitro	ND	ND
16.	Acarbose		100.00	58±0.015

Table 3: Docking Scores, rmsd and interacting residues for the titled norfloxacin derivatives 3i and 3j.

Compound	Docking Score		
	Docking score	rmsd	Interacting residues
Norfloxacin	-14.009	1.105	Arg(526)
3i	-12.5939	1.6177	Arg(526), Asp(542), Asp(327)
3j	-16.658	2.809	Arg (526), Asp(327)
Acarbose	-16.279	1.791	Asp(A203), Asp (542), Asp(A327), His (600), Thr (205)

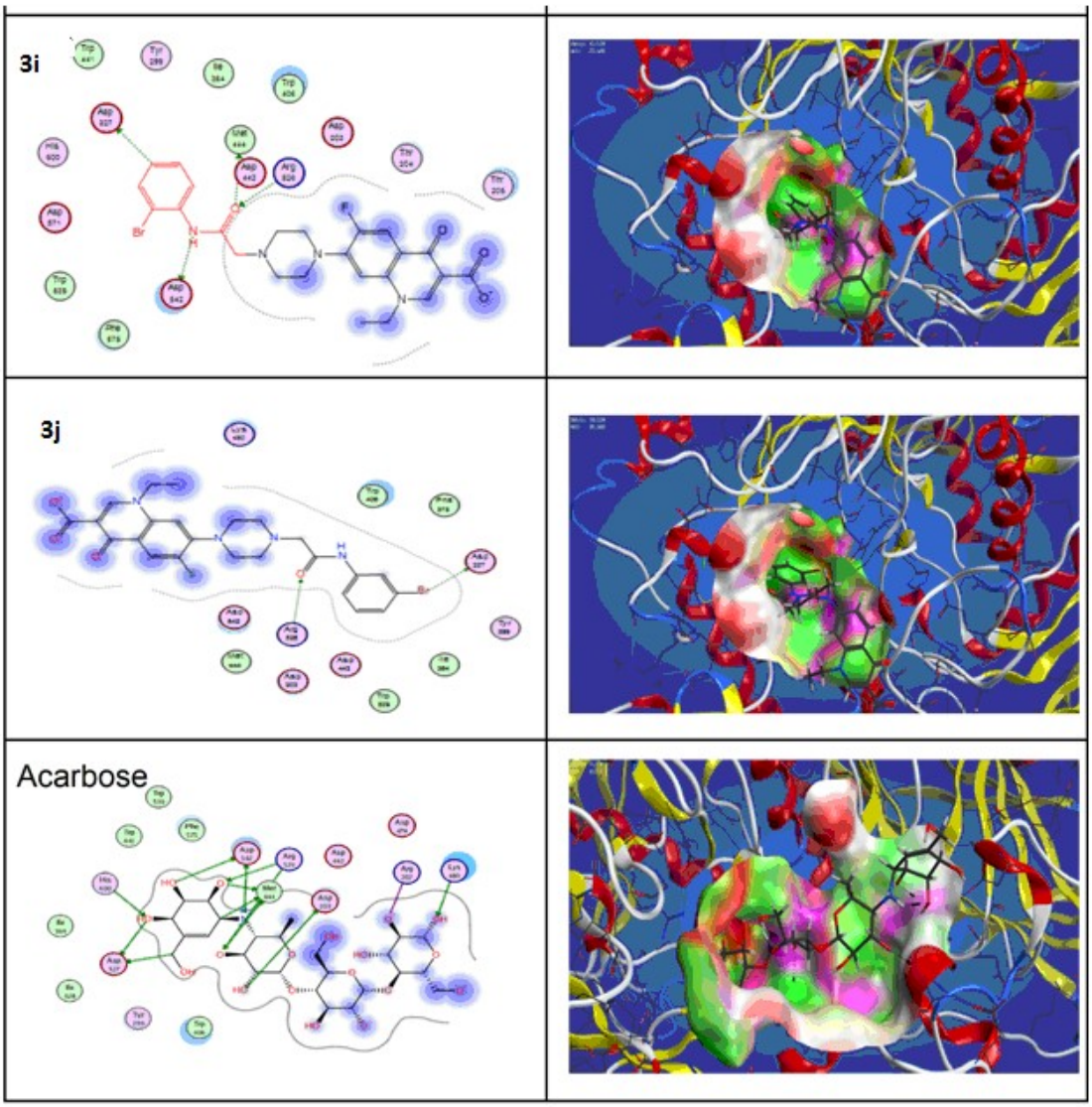


Fig. 1: 2D and 3D interaction modes of most potent compounds.

DISCUSSION

Chemistry

The LCMS studies were quite useful in the confirmation of molecular formula as the calculated MS values matched the found ones. The methylene group of acetamide side chain appeared as singlet ~3.80ppm in ^{1}H NMR and the CON*H* proton was observed at ~8.95ppm. The eight protons of piperazine ring splitted in two groups; one appeared at ~2.52ppm as singlet for 4H while the other 4H at ~4.23ppm.

Inhibitory activity of the synthesized compounds ranges from IC_{50} = 5.55±0.012-128.75±0.104 µM and some of the tested compounds exhibited potent α-glucosidase inhibitory activity compared to the standard drug acarbose. Among the series, compound 3j with IC_{50} = 5.55±0.012 µM was found to be the most active

compound and had the highest inhibitory effect among all the compounds. As shown in table 2, norfloxacin and its derivative 3i showed potent inhibitory activity against α-glucosidase with IC_{50} values of 7.95±0.018 and 7.81± 0.038 µM respectively compared to acarbose (IC_{50} = 58± 0.015 µM).

Fluoroquinolone family of antibiotics is known for reducing blood sugar levels and therefore, this capability should be considered for treatment of individuals with diabetes (Gharib *et al.*, 2020; Granados *et al*., 2018; El Ghandour and Azar, 2015; Saad *et al*., 2021; Okpana and Akah, 2018; Berhe *et al*., 2019). Compounds 3a, 3b, 3g, 3h and 3n displayed very mild alpha-glucosidase inhibition ≥62 µM as compared to norfloxacin with IC_{50} value of 7.95±0.018 µM. Thus, the current study may lead to improve effectiveness of norfloxacin and related antibiotics.

In silico screening

Considering the results obtained from *in vitro* α-glucosidase inhibitions of the carboxamides it was thought worthy to perform molecular docking studies by substantiating the *in-silico* studies. fig. 1 shows the 2D and 3D interaction modes of most potent compounds 3i and 3j with reference to acarbose. In molecular docking studies, it was observed that norfloxacin exhibit docking score -14.009, rmsd 1.105 and bind with only one receptor (Arg526). Compound 3i having 2-bromo R group has docking score -12.593, rmsd 1.617 and bind with three residues (Arg (526), Asp (542), Asp (327). Similarly, compound 3j having bromine at 3 position binds with two residues Arg (526) and Asp (327) with docking score and rmsd -16.658 and 2.809 respectively as shown in table 3.

Ligand-protein interactions were found out via molecular docking of 3a-n compounds with alpha-glucosidase enzyme. Among all compounds, norfloxacin, 3i and 3j have lowest docking score lowest rmsd (-14.009, -12.593 and -16.658), (1.105, 1.617, 2.809) respectively. Acarbose has rmsd value of 1.791 and all the selected compounds have lower rmsd as compared to acarbose that indicates its high binding with the receptor molecule. Ligand molecule binds with receptor molecule by Arg(526), Asp(542), Asp(327) as shown in table 3.

From the *in vitro* α-glucosidase inhibition activity of norfloxacin based acetanilides, it was furthermore observed that among all compounds, 3j having bromine atom at 3 position showed high efficiency at lower concentration (5.55±0.012µM).

CONCLUSION

In this research work, we have reported the synthesis of new norfloxacin and acetanilides hybrids as antidiabetic agents. The synthetic route for norfloxacin based acetamides was developed. The study indicates the potential of fluoroquinolone ring system to act as a template for new antidiabetic agents. Interestingly, compound 3i and 3j (having 2-Br and 3-Br substitution) emerged as potent alpha-glucosidase inhibitors with much better efficacy than acarbose. Overall, this work could act as a lead for the discovery of new antimicrobial and antidiabetic agents based on norfloxacin type of molecules.

ACKNOWLEDGEMENT

The author KW, UAA & MA acknowledge the support from 'Higher Education Commission of Pakistan' and 'Government College University, Faisalabad' for financial support. SS acknowledges University Teknologi MARA for the financial support under grant number 600-IRMI 5/3 LESTARI (025/2019).

REFERENCES

Adhikary KK and Lee HW (2011). Nucleophilic substitution reactions of N-methyl α-Bromoacetanilides with benzylamines in dimethyl sulfoxide. *Bull. Korean Chem. Soc.,* **32**(3): 857-862.

Akhtar R, Zahoor AF, Rasul A, Khan SG and Ali KG (2021). *In-vitro* cytotoxic evaluation of newly designed ciprofloxacinoxadiazole hybrids against human liver tumor cell line (Huh7). *Pak. J. Pharm. Sci.*, **34**(3): 1143-1148.

Berhe A, Russom M, Bahran F and Hagos G (2019). Ciprofloxacin and risk of hypolycemia in non-diabetic patients. *J. Med. Case Rep.*, **13**(1): 142.

Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW and Malanda B (2018). IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Res. Clin. Pract.,* **138**: 271-281.

Dash RP, Babu RJ and Srinivas NR (2018). Reappraisal and perspectives of clinical drug-drug interaction potential of α-glucosidase inhibitors such as acarbose, voglibose and miglitol in the treatment of type 2 diabetes mellitus. *Xenobiotica.,* **48**(1): 89-108.

El Ghandour S and Azar ST (2015). Dysglycemia associated with quinolones. *Prim. Care Diabetes*, **9**(3): 168-171.

Gharib HM, Abajy MY and Omaren A (2020). Investigating the effect of some fluoroquinolones on blood glucose and insulin levels in STZ-induced diabetic wistar rats. *Res. J. Pharm. Technol.*, **13**(12): 5993-5998.

Granados J, Ceballos M and Amariles P (2018). Hypo or hyperglycemia associated with fluoroquinolone use. *Rev. Med. Chile.*, **146**(5): 618-626.

Inc CCG (2016). Molecular operating environment (MOE). Chemical Computing Group Inc 1010

Sherbooke St. West, Suite# 910, Montreal, QC, Canada, H3A 2R7.

Jimenez-Montero JG. 1616-P: Mortality rates due to diabetes mellitus in Costa Rica, 2007-2017. Am Diabetes Assoc2019.

Kulkarni S, Agarwal DK and Pallavi A (2017). Development of a novel method for the analysis of the drug in low concentration using an antidiabetic drug 'Voglibose' for the study. *J. Pharm. Res. Int.,* **19**(2): 1-9.

Mohammadi-Khanaposhtani M, Rezaei S, Khalifeh R, Imanparast S, Faramarzi MA, Bahadorikhalili S, Safavi M, Bandarian F, Esfahani EN, Mahdavi M and Larijani B (2018). Design, synthesis, docking study, α-glucosidase inhibition and cytotoxic activities of acridine linked to thioacetamides as novel agents in treatment of type 2 diabetes. *Bioorg. Chem.,* **80**: 288-295.

Okpana FO and Akah PA (2018). Effect of Diabetes on the Pharmacokinetic Profile of Fluoroquinolones. *Int. J. Med. Pharm.*, **6**(2): 21-27.

Oniga SD, Palage MD, Araniciu C, Marc G, Oniga O, Vlase L, Prisacari V, Valica V, Curlat S and Uncu L (2018). Design, synthesis, molecular docking and antibacterial activity evaluation of some novel norfloxacin analogues. *Farmacia,* **66**(6): 1048-1058.

Qian D, Zhang T, Zheng P, Liang Z, Wang S, Xie J, Zhao L, Zhang Y and Situ B (2018). Comparison of oral antidiabetic drugs as add-on treatments in patients with type 2 diabetes uncontrolled on metformin: a network meta-analysis. *Diabetes Ther.*, **9**(5): 1945-1958.

Saad NA, Elberry AA, Samy Matar H and Hussein RR (2021). Effect of ciprofloxacin vs levofloxacin on QTc‐interval and dysglycemia in diabetic and non‐diabetic patients. *Int. J. Clin. Pract.*, **75**(5): e14072.

Sabo A, Tomas A, Tomic N, Mikov M, Horvat O, Popovic R and Tomic Z (2015). Pharmacokinetic/ pharmacodynamic based dosing of ciprofloxacin in complicated urinary tract infections. *Bangladesh J. Pharmacol.,* **10**(3): 621-626.

Saddique FA, Ahmad M, Ashfaq UA, Ahmad MN, Anjum MN, Mohsin NUA and Aslam S (2019). Alpha-glucosidase inhibition and molecular docking studies of 1, 2-benzothiazine 1, 1-dioxide based carbo-hydrazides. *Pak. J. Pharm. Sci.*, **32**(6 Supp), 2829-2834.

Sohretoglu D, Sari S, Ozel A and Barut B (2017). α-Glucosidase inhibitory effect of *Potentilla astracanica* and some isoflavones: Inhibition kinetics and mechanistic insights through *in vitro* and *in silico* studies. *Int. J. Biol. Macromol.,* **105**: 1062-1070.

Sriram D, Yogeeswari P, Senchani G and Banerjee D (2007). Newer tetracycline derivatives: Synthesis, anti-HIV, antimycobacterial activities and inhibition of HIV-1 integrase. *Bioorg. Med. Chem. Lett.*, **17**(8): 2372-2375.

Suresh N, Nagesh HN, Renuka J, Rajput V, Sharma R, Khan IA and Gowri CSKV (2014). Synthesis and evaluation of 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-(2-(4-substitutedpiperazin-1-yl) acetyl) piperazin-1-yl) quinoline-3-carboxylic acid derivatives as anti-tubercular and antibacterial agents. *Eur. J. Med. Chem.,* **71**: 324-332.

Taj S, Ashfaq UA, Aslam S, Ahmad M and Bhatti SH (2019). Alpha-glucosidase activity of novel pyrazolobenzothiazine 5, 5-dioxide derivatives for the treatment of diabetes mellitus. *In vitro* combined with molecular docking approach. *Biologia,* **74**(11): 1523-1530.

Teyhoo M, Hosseini F, Ardestani MS and Ghorbani M (2020). Synthesis and evaluation of a novel nanosized anionic linear globular dendrimer G2-ciprofloxacin conjugate against prostate cancer. *Pak. J. Pharm. Sci.*, **33**(6): 2589-2594.

Walayat K, Ahmad M, Rasul A, Aslam S, Anjum MN, Sultan S and Iqbal M (2020). Synthesis, characterization and antimicrobial activity of norfloxacin based acetohydrazides. *Pak. J. Pharm. Sci.*, **33**(2): 855-860.

Yadav V and Talwar P (2019). Repositioning of fluoroquinolones from antibiotic to anti-cancer agents: An underestimated truth. *Biomed. Pharmacother.,* **111**: 934-946.

Zhang B, Watt JM, Cordiglieri C, Dammermann W, Mahon MF, Flügel A, Guse AH and Potter BV (2018). Small molecule antagonists of NAADP-induced Ca 2+ release in T-Lymphocytes suggest potential therapeutic agents for autoimmune disease. *Sci. Rep.*, **8**(1): 1-17.

Zhang GF, Liu X, Zhang S, Pan B and Liu ML (2018). Ciprofloxacin derivatives and their antibacterial activities. *Eur. J. Med. Chem.*, **146**: 599-612.