

**Research Article**

**In Silico Molecular Modeling of Chalcone Based Aryloxyethylamines as Antihyperglycemic Agents**

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**Abstract**

Despite enormous efforts have been made in the search for new drugs, diabetes mellitus (DM) still remains the cause of mortality worldwide. A series of chalcone based aryloxyethylamines were synthesized and evaluated for their anti-hyperglycemic activity in SLM and STZ rat models. The majority of the compounds exhibited better metabolic profile compared to the commercial antidiabetic drug, Troglitazone. The anti-glycemic activity was found to be dependent upon the structure of the aryloxyethylamines. A definite structure–activity relationship was observed while varying the nature and position of the different amines in ring-A of chalcones.

**Keywords:** Chalcone; Antihyperglycemic; Aryloxyethylamine; Oxidative stress; Antioxidant

**Introduction**

Diabetes mellitus, an assembly of metabolic diseases in which the patient has elevated blood glucose, either because of insufficient insulin production or because the proper non-responsibility of body’s cells to insulin, or both. NIDDM is a chaos characterized by insulin resistance, hyperglycemia and hyperinsulinaemia, frequently associated with obesity, dyslipidemia and hypertension foremost to cardiovascular risks [1]. In such cases, compensation of normal adipose tissue levels argues in mitigation of the insulin resistant state [2]. Diminution of body fat growth via diet and exercise is generally the first treatment for diabetes with the link between obesity and type-II diabetes. The anti-obesity drugs based therapies for NIDDM, are targeted at a lessening of energy intake or absorption (anorectic drugs) but an increase in energy expenditure (thermogenic drugs) serve as a gorgeous substitute for the treatment of obesity and hence, diabetes. Induction of thermogenesis is either through stimulation of nuclear receptor of PPAR family [3] or adrenergic receptor (AR) in membrane of adipose tissue [4].

In 1980s, β3-adrenergic receptor was revealed on the cell surface of both white and brown adipose tissues. β3-AR agonists were observed to concurrently augment lipolysis, fat oxidation, energy outlay [5] and insulin action foremost to the belief that, this receptor might aﬀord as an attractive target for the treatment of diabetes and obesity [6]. Arylethanolamines and arylpropanolamines were first reported as β3-AR agonists earlier [7]. CL-316,243 is the most selective β3-AR agonist tested [8].

Troglitazone is also an antidiabetic drug of thiazolidinedione (I) class having unique structural feature [9], (Figure 1) is the presence of an antioxidant moiety of vitamin-E mutually with a PPAR-γ nuclear receptor motivating pharmacophore in a single molecule. It is however failed with toxic metabolites but provided a source for further scheming molecules for multi-factorial diseases. Oxidative stress plays a vital role in diabetic patients by macrovascular complications through Maillard reaction [10]. The chalcones with antioxidant properties having plant origin have attracted our attention to explore hybrid structures with them for antidiabetic activity. Chalcones are natural products having various biological activities including antioxidant [11], antimalarial [12], antileishmanial [13], anti-inflammatory [14] and antitumor [15]. The antioxidative nature of chalcones might be one of the factors for different activities. In this ongoing programme, we have synthesized two series of chalcone based arylpropanolamines (II) [16] (Figure 1) have shown a potent anti-diabetic activity.

![Figure 1: Prototypes of thiazolidinones and chalcones.](image1)

![Figure 2: Prototype structure of synthesized molecules.](image2)

![Scheme 1: Reagents: (i) Aq. NaOH, methanol, r. t., 6-8 hr; (ii) N, N-diethyl-N-chloroformamide, dried K2 CO3, dry acetone.](image3)
| Compounds | Chemical Structure | % Improvement SLM | % Lowering (STZ) |
|-----------|-------------------|------------------|-----------------|
|           |                   | 5 hr  | 24 hr |                   |
| 1         | ![Chemical Structure](1) | +17.5 | ND    | ND               |
| 2         | ![Chemical Structure](2) | 2.65  | ND    | ND               |
| 3         | ![Chemical Structure](3) | 23.8* | 5.63  | 17.6*            |
| 4         | ![Chemical Structure](4) | 17.2  | ND    | ND               |
| 5         | ![Chemical Structure](5) | 25.2* | 11.7  | 14.0             |
| 6         | ![Chemical Structure](6) | 11.3  | ND    | ND               |
| 7         | ![Chemical Structure](7) | 21.3* | 20.8**| 18.0**           |
| 8         | ![Chemical Structure](8) | 18.8* | 13.4  | 6.55             |
| 9         | ![Chemical Structure](9) | 20.5* | 14.8* | 9.71             |
| 10        | ![Chemical Structure](10) | 19.1* | 11.0  | 12.2             |
| 11        | ![Chemical Structure](11) | 18.0* | 14.8* | 20.4*            |
| 12        | ![Chemical Structure](12) | 20.7* | 21.9* | 23.2*            |
| 13        | ![Chemical Structure](13) | 25.8**| 17.9* | 16.0*            |
| 14        | ![Chemical Structure](14) | 24.9**| 27.6**| 23.1**           |
| 15        | ![Chemical Structure](15) | 18.5* | 15.0* | 22.6*            |
| 16        | ![Chemical Structure](16) | 22.8**| 14.6* | 14.3*            |
| 17        | ![Chemical Structure](17) | 19.6* | 29.4**| 25.0**           |
compounds of this new series have shown better anti-hyperglycemic activity than earlier series.

Chemistry

Synthesis of chalcone derived, ring-A substituted aryloxyethylamines (1-20)

The chalcones were synthesized with the help of Claisen-Schmidt condensation, which has been reported above. 4-hydroxy-acetophenone (A) and appropriate amount of substituted benzaldehydes (B1-B7) were reacted using aqueous sodium hydroxide in methanol at room temperature to provide corresponding hydroxy-chalcones (C1-C7). These chalcones were reacted with N,N-dialkylaminoethylchloride in presence of dried K2CO3 (4-5eq.) in acetone to get products 1-20 (Scheme 1).

Results and Discussion

Anti-hyperglycemic activity of chalcone derived aryloxyethylamines

These molecules were evaluated for anti-hyperglycemic activity on both SLM and STZ models which have shown moderate to good potential as anti-hyperglycemic agents presented below (Table 1).

Effect of compounds and standard drug metformin on improvement of glucose tolerance on normal rats (SLM)

A set of 20 substituted chalcones with different functionalities at R1 and R2 were synthesized in the Medicinal and Process Development Chemistry of the Institute. These were first evaluated for improvement in glucose tolerance in normal rats. The results obtained from the variance analysis showed in Table 1, depict the percentage improvement profile of 20 test compounds (1-20) and standard drug Metformin. Among these 20 compounds, most of the compounds i.e., 3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 showed significant effect were calculated to be around 35.6**% in glucose tolerance in normal rats. The results obtained from these molecules were evaluated for anti-hyperglycemic activity evaluation. Among the tested compounds i.e., 3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 and 20 compound code 7, 9, 11, 12, 13, 14, 15, 16, 17, 19 and 20 caused significant decline of 20.8, 14.8, 14.8, 21.9, 17.9, 27.6, 15.0, 14.4, 29.1, 34.1, 17.1% during 5 hr and 18.0, 9.71, 20.4, 23.2, 16.0, 23.1, 22.6, 14.3, 25.0 and 9.74% during 24 hr respectively whereas Compound code 3, 5, 6, 8, 10 and 18 showed very mild effect on STZ treated rats. The standard drug Metformin at the dose level of 100 mg/kg showed a blood glucose lowering effect of 28.9% and 25.7% after 5 hr and 24 hr intervals respectively.

Experimental Data

Chemistry

4’-(2-Dimethylamino-ethoxy)-3’, 4-methinedioxy-chalcone (1): To a solution of 4-hydroxy-chalcone E1 (0.5 g, 1.86 mmol) in dry acetonitrile was added dried K2CO3 (3eq.) and 2-chloroethyl-dimethylamine (0.22 g, 2.14 mmol). This reaction mixture was refluxed for 7-8 hrs at 70°C to get 14. Solid, Yield: 27%, m. p.: 103°C, MS: 340 (M+1), IR(KBr): 3415, 1600, 449, 1HNMR (300 MHz, CDCl3), δ 7.15 (d, J = 11.25 Hz, 2H 2’, 6’–H), 7.73 (d, J = 15.51 Hz, 1H, α–H), 7.39 (d, J = 15.48 Hz, 1H, α–H), 7.17 (s, 1H, 2-H), 7.13 (d, J = 8.01 Hz, 1H, 6-H), 7.00 (d, J = 8.76 Hz, 2H, 3’, 5’-H), 6.85 (d, J = 8.01 Hz, 1H, 5-H), 6.03 (s, 2H, OCH3), 4.15 (t, 2H, OCH2), 2.769 (t, 2H, -NCH2), 1.62, ( s, 6H, -NCH2) 1.87-1.82 (m, 4H, CH2.

4’-(2-Diethylamino-ethoxy)-3’, 4-methinedioxy-chalcone (2): To a solution of 4-hydroxy-chalcone E1 (0.5 g, 1.86 mmol) in dry acetonitrile was added dried K2CO3 (3eq.) and 2-chloroethyl-diethylamine (0.25 g, 2.18 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 15. Solid, Yield: 5%, m. p.: 105°C, MS: 368 (M+1), IR(KBr): 3020, 1516, 476, 1HNMR (300 MHz, CDCl3), δ 8.02 (d, J = 8.82 Hz, 2H 2’, 6’–H), 7.73 (d, J = 15.52 Hz, 1H, β–H), 7.38 (d, J = 15.50 Hz, 1H, α–H), 7.13 (s, 1H, 2-H), 7.13 (d, J = 8.08 Hz, 1H, 6-H), 6.15 (d, J = 8.63 Hz, 2H, 3’, 5’-H), 5.68 (d, J = 7.15 Hz, 1H, 5-H), 6.03 (s, 2H, OCH3), 4.13 (t, 2H, OCH2), 2.7 (t, 2H, -NCH2), 2.27 (q, 2H, CH2), 1.62, ( s, 6H, -NCH2).

4’-(2-Pyrolidin-1-yl-ethoxy)-3’, 4-methinedioxy-chalcone (3): To a solution of 4-hydroxy-chalcone E1 (0.3 g, 1.12 mmol) in dry acetonitrile was added dried K2CO3 (3eq.) and 2-chloropyrrolidinone (0.15 g, 1.25 mmol). This reaction mixture was refluxed for 9-10 hrs at 70°C to get 16. Solid, Yield: 73.17%, m. p.: 140°C, MS (ESI): 366 (M+1), IR (KBr): 3380, 2361, 1515, 416, 670, 1HNMR (300 MHz, CDCl3), δ 8.03 (d, J = 8.79 Hz, 2H 2’, 6’–H), 7.74 (d, J = 15.54 Hz, 1H, β–H), 7.40 (d, J = 15.54 Hz, 1H, α–H) 7.19 (s, 1H, 2-H), 7.14 (d, J = 8.07 Hz, 1H, 6-H), 7.01 (d, J = 8.83 Hz, 2H, 3’, 5’-H), 6.84 (d, J = 8.61 Hz, 1H, 5-H), 6.05 (s, 2H, OCH3), 4.19 (t, 2H, OCH2), 2.11 (t, 2H, -NCH2), 2.67-2.27 (m, 4H, -NCH2).
4'- (2- Piperidin-1-yl-ethoxy)-3, 4- methylenedioxy- chalcone (4): To a solution of 4-hydroxy-chalcone E 1 (0.3 g, 1.12 mmol) in dry acetone was added dried K2CO3 (3eq.) and 2-chloroethyl-diethylamine (0.27 g, 2.05 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 18. Solid, Yield: 80.73%, m. p.: 28-30°C, MS (ESI): 370 (M+1), IR (KBr): 3433, 1640, 64, 1HNMR (300 MHz, CDCl3), δ 8.04 (d, J = 8.85 Hz, 2H, 2', 6'-H), 7.76 (d, J = 15.60 Hz, 1H, α–H), 7.59 (d, J = 8.46 Hz, 2H, 2', 6-H), 7.77 (d, J = 15.66 Hz, 1H, β–H), 5.79 (d, J = 8.46 Hz, 2H, 2', 6-H), 7.53 (d, J = 5.5 Hz, 1H, α-H), 7.40 (d, J = 8.46 Hz, 2H, 3', 5-H), 7.01 (d, J = 8.85 Hz, 2H, 3', 5-H), 4.19 (t, 2H, OCH2), 3.20 (t, 2H, NCH3), 2.52 (m, 4H, NCH2), 1.70-1.27 (m, 8H, -CH2).

4'- (2- Dimethoxyethoxy)-3, 5- dichloro- chalcone (7): To a solution of 4-hydroxy-chalcone E 4 (0.5 g, 2.11 mmol) in dry acetone was added dried K2CO3 (5eq.) and 2-chloroethyl-morpholine (0.168 g, 2.24 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 25. Solid, Yield: 77.43%, MS (ESI): 386 (M+1), IR (Neat): 3414, 1605, 64, 1HNMR (300 MHz, CDCl3), δ 8.03 (d, J = 8.85 Hz, 2H, 2', 6'-H), 7.78 (d, J = 15.68 Hz, 1H, α–H), 7.59 (d, J = 8.46 Hz, 2H, 2', 6-H), 7.76 (d, J = 15.60 Hz, 1H, β–H), 7.59 (d, J = 8.46 Hz, 2H, 2', 6-H), 7.53 (d, J = 5.5 Hz, 1H, α-H), 7.40 (d, J = 8.46 Hz, 2H, 3', 5-H), 7.01 (d, J = 8.85 Hz, 2H, 3', 5-H), 4.19 (t, 2H, OCH2), 3.20 (t, 2H, NCH3), 2.52 (m, 4H, NCH2), 1.70-1.27 (m, 8H, -CH2).

4'- (2- Diethylaminoethoxy)-3, 4- methylenedioxy-chalcone (6): To a solution of 4-hydroxy-chalcone E 3 (0.5 g, 2.11 mmol) in dry acetone was added dried K2CO3 (4eq.) and 2-chloroethyl-azepane (0.29 g, 1.35 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 21. Solid, Yield: 74.63%, m. p.: 107-27°C, MS (ESI): 391 (M+1), IR (KBr): 3415, 1605, 64, 1HNMR (300 MHz, CDCl3), δ 8.03 (d, J = 8.85 Hz, 2H, 2', 6'-H), 7.78 (d, J = 15.60 Hz, 1H, α–H), 7.59 (d, J = 8.46 Hz, 2H, 2', 6'H), 7.76 (d, J = 15.60 Hz, 1H, β–H), 7.59 (d, J = 8.46 Hz, 2H, 2', 6-H), 7.53 (d, J = 5.5 Hz, 1H, α-H), 7.40 (d, J = 8.46 Hz, 2H, 3', 5-H), 7.01 (d, J = 8.85 Hz, 2H, 3', 5-H), 4.19 (t, 2H, OCH2), 3.20 (t, 2H, NCH3), 2.52 (m, 4H, NCH2), 1.70-1.27 (m, 8H, -CH2).

4'- (2- Piperidin-1-yl-ethoxy)-3, 4- methylenedioxy-chalcone (8): To a solution of 4-hydroxy-chalcone E 1 (0.5 g, 2.11 mmol) in dry acetone was added dried K2CO3 (3eq.) and 2-chloroethyl-piperidine (0.29 g, 2.24 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 22. Solid, Yield: 88.23%, m. p.: 48-50°C, MS (ESI): 358 (M+1), IR (KBr): 3378, 1640, 64, 1HNMR (300 MHz, CDCl3), δ 8.03 (d, J = 8.85 Hz, 2H, 2', 6'-H), 7.78 (d, J = 15.60 Hz, 1H, α–H), 7.59 (d, J = 8.46 Hz, 2H, 2', 6-H), 7.76 (d, J = 15.60 Hz, 1H, β–H), 7.59 (d, J = 8.46 Hz, 2H, 2', 6-H), 7.53 (d, J = 5.5 Hz, 1H, α-H), 7.40 (d, J = 8.46 Hz, 2H, 3', 5-H), 7.01 (d, J = 8.85 Hz, 2H, 3', 5-H), 4.19 (t, 2H, OCH2), 3.20 (t, 2H, NCH3), 2.52 (m, 4H, NCH2), 1.70-1.27 (m, 8H, -CH2).
4'-2-Diethylamino-ethoxy)-4-nitro-chalcone (16): To a solution of 4-hydroxy-chalcone E$_5$ (0.5 g, 1.85 mmol) in dry acetone was added dried K$_2$CO$_3$ (4eq.) and 2-chloroethyl-diethylamine (0.26 g, 2.32 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 30. Solid, Yield: 78.74%, m. p.: 12-14°C, MS (ESI): 364 (M+1), IR (KBr): 3422, 1753, 1380, 1013, 694 cm$^{-1}$. 

4'-2-Piperidin-1-yl-ethoxy)-4-nitro-chalcone (17): To a solution of 4-hydroxy-chalcone E$_5$ (0.5 g, 1.85 mmol) in dry acetone was added dried K$_2$CO$_3$ (4eq.) and 2-chloroethyl-piperidine (0.3 g, 2.19 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 31. Solid, Yield: 83.80%, m. p.: 42°C, MS (ESI): 381 (M+1), IR (KBr): 211, 1753, 74, 65, 17, 6, 5, 1, 8.92 (d, J = 8.76 Hz, 2H, 3, 5-H), 8.06 (d, J = 8.85 Hz, 2H 2', 6'-H) 7.83 (d, J = 15.69 Hz, 1H, 1', β-H), 7.80 (d, J = 8.70 Hz, 2H, 2, 6-H), 7.67 (d, J = 15.5 Hz, 1H, α-H), 7.03 (d, J = 8.85 Hz, 2H, 3', 5'-H) 4.22 (t, 2H, OCH$_2$), 2.83 (t, 2H, NCH$_2$), 2.54-23 (m, J = 8.70 Hz, 2H, 2, 6-H), 7.64 (d, J = 15.7 Hz, 1H, α-H), 7.01 (d, J = 8.82 Hz, 2H, 3', 5'-H) 4.19 (t, 2H, OCH$_2$), 3.02 (t, 2H, NCH$_2$), 2.83-2.80 (m, 4H, NCH$_2$), 1.67-1.60 (m, 6H, CH$_2$).

4'-2-Azetap-1-yl-ethoxy)-4-nitro-chalcone (18): To a solution of 4-hydroxy-chalcone E$_5$ (0.5 g, 1.85 mmol) in dry acetone was added dried K$_2$CO$_3$ (4eq.) and 2-chloroethyl-azepane (0.31 g, 2.26 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 32. Solid, Yield: 75.34%, m. p.: 14-16°C, MS (ESI): 366 (M+1), IR (Neat): 3318, 2930, 1753, 1673, 1516, 1378, 1303, 1024, 761 cm$^{-1}$. 

4'-2-Diethylamino-ethoxy)-2-chloro-chalcone (19): To a solution of 4-hydroxy-chalcone E$_5$ (0.5 g, 1.27 mmol) in dry acetone was added dried K$_2$CO$_3$ (3eq.) and 2-chloroethyl-diethylamine (0.3 g, 2.26 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 33. Liquid, Yield: 75.34%, MS (ESI): 358 (M+1), IR (Neat): 3148, 478, 1HNMR (300 MHz, CDCl$_3$), δ 7.80 (d, J = 15.60 Hz, 1H, β-H) 7.56 (d, J = 8.34 Hz, 2H, 2, 6-H), 7.52 (d, J = 15.81 Hz, 1H, α-H), 7.24 (d, J = 7.9 Hz, 2H, 3, 5-H), 7.00 (d, J = 8.85 Hz, 2H, 3', 5'-H) 4.18 (t, 2H, OCH$_2$), 3.01 (t, 2H, NCH$_2$), 2.82 (m, 4H, NCH$_2$), 2.41 (s, 3H, CH$_3$), 1.69-1.27 (m, 8H, CH$_2$).

4'-2-Piperidin-1-yl-ethoxy)-4-methoxy-chalcone (20): To a solution of 4-hydroxy-chalcone E$_5$ (0.5 g, 1.13 mmol) in dry acetone was added dried K$_2$CO$_3$ (3eq.) and 2-chloroethyl-piperidine (0.3 g, 2.19 mmol). This reaction mixture was refluxed for 8-9 hrs at 70°C to get 34. Liquid, Yield: 73.43%, MS (ESI): 364 (M+1), IR (Neat): 3148, 478, 1HNMR (300 MHz, CDCl$_3$), δ 8.08 (d, J = 15.66 Hz, 1H, β-H), 7.62 (d, J = 8.64 Hz, 2H, 2', 6'-H), 7.40 (d, J = 15.63 Hz, 1H, α-H), 7.59-7.56 (m, 2H, 4, 5-H), 7.41 (s, 1H, 2-H), 7.14 (dd, J = 2.1, 8.07 Hz, 1H, 5-H) 6.13 (d, J = 8.76 Hz, 2H, 3', 5'-H) 4.20 (t, 2H, OCH$_2$), 3.88 (s, 3H, OCH$_3$), 2.82 (t, 2H, CH$_2$N$_2$), 2.55 (m, 4H, NCH$_2$), 1.27-1.60 (m, 4H, CH$_2$), 1.51-1.45 (m, 2H, CH$_2$)
on glucose tolerance or anti-hyperglycemic activity. The results are expressed as mean ± SEM and statistical study was carried out to determine the level of significance by one-way ANOVA followed by Dunnett’s test by graph pad prism software package. It is denoted by p values. Statistically significant differences were put at following levels * represents p< 0.05, ** represents p< 0.01.

Quantitative structure activity relationship

The calibration set and the prediction set: Chalcone based Aryloxyethylamines as Antihyperglycemic agents (Table 1) were included in the calibration set. The Antihyperglycemic activity was expressed by means of the equation A=−log(activity). In this mentioned study it has been also observed that A series of chalcone aryloxyethylamines of type 1-16 (Table 1) act as their anti-hyperglycemic activity, with variable efficacies, depending on their chemical structure. The prediction set contains 13 other not yet synthesized substituted of chalcone aryloxyethylamines, having unknown observed values of activity (Table 1) and structure presented in Figure 2. The discovery of novel bioactive molecule is the primary goal of computational drug discovery. The discovery of novel bioactive molecule is the primary goal of computational drug discovery. The virtual constructions of the molecules and geometry optimization have been done using the Molecular Mechanics force field MM+ of Hyper Chem software [17] (version 7.52, HyperCube Inc. Florida USA). The MM+ force field is an extension of latest MM2 force field which was developed by Allinger and co-workers [18,19]. Separately, for each molecule, 3224DRAGON [20] (version 5.5, Talete srl, Milano, Italy) descriptors have been calculated. Several criteria were used to reduce the DRAGON descriptors while optimizing the information content of the descriptors set. First, descriptors for which no value was available for all the compounds were disregarded. Second, descriptors of which the value is constant (or near-constant) inside each group of descriptors were excluded. After these automatic screening procedures, a set of 1598 DRAGON descriptors was obtained for further analysis. The statistical calculations used for obtaining the QSAR equations were done with PRECLAV as reported earlier [21-23].

Descriptor calculation and quality of the model: The program PRECLAV (8.) computes type (2) multinlinear QSARs.

\[
A=C_0+\sum C_i \cdot D_i
\]

where, A is the (value of) activity; C_i is the free term intercept; C_i are coefficients (weighting factors); D_i are the (value of) significant descriptors; k is the number of descriptors.

The square of Pearson linear correlation \( r^2 \) of observed/computed values, the Fisher function \( F \), the standard error of estimation \( \text{SEE} \), and the quality function \( Q^2 \) are criteria for the quality of prediction for the molecules in calibration set.

\[
F=r^2/(1-r^2) \cdot (N-p)/p
\]

\[
\text{SEE}=[(\sum \Delta^2)/(N-1)]^{1/2}
\]

\[
Q=r^2 \cdot (1-\text{SEE}^2)\cdot N/N
\]

where: \( p \) is number of descriptors; \( N \) is number of molecules in the calibration set; \( \Delta \) is difference \( A_{obs} - A_{calc} \). The descriptors included in the best (by Q function) QSAR are named ‘predictors’. The relative utility of predictors is computed by the formula (6).

\[
U=(R^2-r^2)/(1-r^2)
\]

where \( R^2 \) is the square of Pearson correlation between the observed values and the computed values (using \( p \) predictors) \( r^2 \) is the square of Pearson correlation between the observed values and the calculated values (using the \( p \)-1 predictors, i.e., the QSAR equation without the analyzed predictor)

After computation of \( U \) for each predictor, the values of \( U \) are normalized by the highest of them (the highest value for \( U \) becomes 1000). The predictors with high enough value of \( U \) (\( U > 500 \)) can be considered ‘with high relative utility’. These predictors are useful because they correlate well with \( A_{obs} \) values and present low correlation with other descriptors.

PRECLAV [24] calculates square of cross-validated correlation \( r^2_{cv} \) using LHO (Leave Half Out) method. However, this usual method is applied after ordering of molecules in calibration set according to the observed values of activity. Therefore, the cross-validated function \( r^2_{cv} \) is a measure of homogeneity of calibration set from the point of view of predictors’ set, i.e., from the point of view of structure-activity relation. A low value (<0.4) of \( r^2_{cv} \) means the QSAR for molecules having high values of activity and the QSAR for molecules having low values of activity include the same descriptors, but very different weighting factors. Actually, the computation of \( r^2_{cv} \) is a very drastic ‘internal validation test’.

Applicability of domain and detection of outliers: A QSAR model can be used for showing new compounds if its domain of application is defined [25,26]. The need to characterize the model applicability domain is also reflected in the OECD guiding principle for QSAR model validation [27,28]. QSAR model should only be used for making predictions of compounds fall within the specified domain may be considered reliable. Extent of extrapolation [29,30] is one simple approach to define the applicability of the domain. It is based on the calculation of the hat diagonal (leverage) \( h \) for each chemical, where the QSAR model is used to predict its activity.

\[
h=\sum x_i^2 (X'X)^{-1} x
\]

In equation 9, \( x_i \) is the descriptor-row vector of the query molecule and \( X \) is the \( k \times n \) matrix containing the \( k \) descriptor values for each one of the \( n \) training molecules. A hat diagonal (leverage) value >3(k+1)/n leverage warning limit is considered large.

Outliers are compounds that are poorly fit by the regression model. Outlying compounds should not be removed unless a good reason for their removal can be given. The variance of the observed residuals is not constant. This makes comparisons among the residuals difficult. One solution is to standardize the residuals [31,32], by dividing by their standard deviations. This gives a set of standardized residuals. The cross-validated LOO standardized residuals is a \( [R_{Student}] \) that has the impact of a single observation removed from the mean square error. A molecule is defined as an outlier in which \( [R_{Student}] ≥ 2 \) [32-34].

To visualize the applicability of domain of a developed QSAR model, William plot was used. In the William plot, \( [R_{Student}] \) versus leverage values (\( h \)) are plotted. This plot could be used for an immediate and simple graphical detection of both the response outliers and structurally influential compounds in a model. It must be noted that compounds with high value of leverage and good fitting in the developed model can stabilize the model. On the other hand, compounds with bad fitting in the developed model may be outliers. Thus, combination of leverage and the [\( R_{Student} \)] could be used for assigning the applicability of domain [35-37].

Results and Discussion

The statistical computations were conducted using the specific
formulas and procedures of PRECLAV program algorithm. Using only the “significant” descriptors, PRECLAV computes ten thousand QSAR type (3) multilinear equations. The quality of the obtained equation is reflected by the value of the Q function and also by values of some usual statistical functions. During the PRECLAV MLR analysis using Dragon descriptor, we observed that the 3-parametric model has the highest value of the Q function for anti-hyperglycemic activity and also has the highest predictive power as follows:

Dependent property: anti-hyperglycemic activity (A)

Molecules number in calibration set: 16

Number of “significant” descriptors in presence of set: 277

\[ A = 4.3733 - 1.0262 \times 0.0466; r_2 = 0.925; F = 53.4748; r_2, CV = 0.8777, r^2_p = 0.85599 \]

 Eig06_A = eigen value n. 6 from augmented edge adjacency mat. weighted by edge degree Edge adjacency indices Eigenvalues (U=1000)

G2p=2nd component symmetry directional WHIM index / weighted by polarizability (U=829)

G2m=2nd component symmetry directional WHIM index / weighted by mass WHIM descriptors Directional descriptors (U= 912), SEE =0.0466; r2 =0.925; F =53.4748; r2, CV =0.8777, r^2_p = 0.85599.

SEE = Standard Error of Estimation, r2 = Pearson square correlation, F = Fisher function, r2CV = Pearson cross validated square correlation (Leave one out method), r^2_p = predictive r2 According to algebraic sign of coefficients in QSAR formula and the value of utility U the main factor in influence on activity value is the Eigenvalues (Eigenvalues of the edge adjacency matrix (Eigk_EA), augmented edge adjacency matrices (Eigk_AEA(w) and unsymmetrical weighted edge adjacency matrices (Eigk_EA(w) are also provided by Dragon and Directional WHIM descriptors [WHIM descriptors (Weighted Holistic Invariant Molecular descriptors) are geometrical descriptors based on statistical indices calculated on the projections of the atoms along principal axes.

WHIM descriptors are built in such a way as to capture relevant molecular 3D information regarding molecular size, shape, symmetry and atom distribution with respect to invariant reference frames)] Play dominant role in activity. The negative correlation of Eig06A shows that, increases this descriptor value decreases the activity and positive correlation of the G2p shows the increase the polarizibility increase the activity.

Validation of the computation procedure

For the validation of the method, we have proceeded to a QSAR study with a validation set and reduced calibration set. The set in bold letter with star containing compound in Table 1 are the most significant is the correlation between the calculated and selected for validation set (comp. no 2,10,12 and 16). The selection of the set should be such that it captures all the features and characteristics of the whole set of molecules validation set was extracted from the homogenized calibration. From the point of view of them considerations discussed here, the experimental values of activity for the molecules in the validation set. In Table 2 and Figure 1 there are listed the calculated values and the experimental values of the anti-hyperglycemic activity power for the molecules in the validation set. We have found good predictive r^2.

Applicability domain: We used |RStudent| of observed inhibitory activity calculated by the obtained models and hat diagonal (leverage) for assigning applicability of domain (AD). Values for leverage have been calculated for both calibration set and prediction set compounds showing in Table 2. Applicability of domain for the developed model

![Figure 3: Effect of synthetic compounds and standard anti-diabetic drug metformin on blood glucose profile of streptozotocin-induced diabetic rats. Values are mean % change in 5 animals of a group; significance: *p<0.05, **p<0.01 when compared with control.](image)

| Comp. No. | Obs. (A) | Est. (A) | Res. A | R Student | Diagonal |
|-----------|---------|---------|--------|-----------|----------|
| 3         | 1.2455  | 1.193   | 0.053  | 1.0871    | 0.113    | A 3.001 |
| 5         | 1.1461  | 1.12    | 0.026  | 0.5578    | 0.2245   | B 2.683 |
| 7         | 1.2553  | 1.305   | -0.05  | -1.031    | 0.1304   | C 2.734 |
| 8         | 0.8162  | 0.858   | -0.042 | -1.0729   | 0.428    | D 2.765 |
| 9         | 0.9872  | 0.952   | 0.035  | 0.8383    | 0.3809   | E 2.422 |
| 10        | 1.0864  | 1.136   | -0.049 | -1.1263   | 0.2783   | F 2.53 |
| 11        | 1.3096  | 1.367   | -0.047 | -1.0226   | 0.2083   | G 2.045 |
| 12        | 1.3655  | 1.288   | 0.078  | 1.669     | 0.0845   | H 2.692 |
| 13        | 1.2041  | 1.239   | -0.035 | -0.6915   | 0.0973   | I 2.052 |
| 14*       | 1.3636  | 1.3     | 0.063  | 1.3873    | 0.1713   | J 2.689 |
| 15        | 1.3641  | 1.397   | -0.043 | -0.9113   | 0.1776   | K 2.283 |
| 16*       | 1.1553  | 1.113   | 0.042  | 0.8367    | 0.0923   | L 2.396 |
| 17        | 1.3979  | 1.372   | 0.026  | 0.8189    | 0.6436   | M 1.835 |
| 18        | 1.1206  | 1.178   | -0.058 | -1.2749   | 0.205    | - - |
| 19        | 1.3979  | 1.395   | 0.003  | 0.0707    | 0.2451   | - - |
| 20*       | 0.9886  | 0.99    | -0.002 | -0.0437   | 0.5196   | - - |

Table 2: Observed anti-hyperglycemic activity and their corresponding A value where A=log, anti-hyperglycemic activity, estimated inhibition constant (A), hat diagonal, standardized residual, |RStudent| of the calibration set molecules of chalcone having good % lowering in STZ (24 hrs) model and predicted value of A-M not yet synthesized compounds.

![Figure 4: Structure of compounds A-M predicted as antihyperglycemic agents.](image)
of calibration set is shown in William plot (Figures 3–7). Influential compounds are points with leverage value higher than the warning leverage limit 1, so outliers are not present. It can be seen in the William plot; all molecules in calibration set lie in the application domain of the developed model. The computed activity of the prediction set compounds is also within limit of hat diagonal.

Conclusions

The compounds with alkylaminoethoxy group on ring-A and electron withdrawing group on ring-B exhibited potential antihyperglycemic activity in SLM and STZ models. In calibration set polarizibility and Mass play dominant role for the activity. Many attempts have been made to design and develop novel drugs against antihyperglycemic activity in SLM and STZ models. In calibration plot; all molecules in calibration set lie in the application domain of the developed model. The computed activity of the prediction set compounds is also within limit of hat diagonal.

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