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Synthesis, Antiglycating and Antioxidant activities of Triazolo hexahydroquinazolines

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Abstract: Some 5-aryl-triazolo[*4,3-a*] hexahydro quinazoline derivatives (**4a-4e & 5a-5e)** were synthesized and evaluated for their *in vitro* antiglycation and antioxidant activities. Title compounds were found to have potent antiglycating activity than rutin in the range between 0.096 and 0.300 µM.The compounds **4b** and **5c** were the most active ones among them. The tested compounds had shown potent to moderate antioxidant activity by DPPH assay than BHT except **5e** (*p*-NO_2 phenyl derivative). Among all, hydroxyl derivatives (**4a-4e)** are more potent than the corresponding thiols (**5a-5e**).

Keywords: Hexahydroquinazolines; Triazole; Antiglycating agents; Antioxidant agents

1. Introduction

The incidence of type-2 diabetes is increasing at an alarming rate and about 1–2% of the world's population is affected with over 100 million diabetic patients worldwide. Diabetes is a multi-factorial disease with associated complications. However, exact molecular basis of these complications is not fully understood yet. Hyperglycemia is one of the contributing factors of these complications i.e. atheroma, hypertension and microangiopathy, mostly attributed to the formation of sugar-derived substances called advanced glycation end products (AGEPs) [1]. A vast number of evidences suggest that AGEPs are important physio-pathogenic mediators of almost all diabetic complications [2]. AGEPs are a group of molecules formed in a non-enzymatic glycation from combination of reducing sugars with free amino groups of proteins, lipids, enzymes, phospholipids and nucleic acids.

Initial product of amino group of protein with carbonyl group of glucose interaction, is a Schiff base, whereas the rearrangement of Schiff base intermediate to Amadori product takes number of days. An Amadori product undergoes subsequent oxidative modifications (glycoxidations) that are induced by reactive oxygen species (ROS) and reactive nitrogen species (RNS). The end result of these complex series of reactions is the formation of AGEs. Extent of glycation of vital biochemicals is dependent on the degree and duration of hyperglycemia *in vivo*. Considerable effort has been focused on the discovery of new inhibitors of glycation because of their therapeutic potential [3].

The antiglycating agents may react with carbonyl group of reducing sugars, Amadori products and 3-deoxyglucosones to prevent AGEPs formation. Certain agents have been established that can cleave AGEP cross-links and perhaps open the possibility of moving back the steady process of diabetic complications [4]. Antioxidants may offer protection against glycated free radicals, while chelators are helpful in removing the transition metals to prevent glycoxidations (auto oxidation) of glucose and Amadori products [5, 6].

Compounds with both antiglycation and antioxidant properties may offer therapeutic potential. It has been found that aged garlic extract (AGE) inhibit the formation of AGEPs *in vitro* and it also prevents the formation of glycation-derived free radicals.S-Allyl-cysteine is a key component of aged garlic extract that acts as a potent antioxidant and may inhibit the AGEPs formation[7]. Aminoguanidine (inhibitor of AGEP formation) was found to prevent retinopathy in diabetic animals and has been withdrawn from phase III clinical trials due to its toxicity, hence can serve as a prototype for novel molecules that are being synthesized and evaluated *in vitro*[8, 9].

Quinazoline and its derivatives constitute an interesting molecule among the most important classes of an hetero aromatic bicyclic compounds with wide-ranging biological and pharmacological activities such analgesic[10], anticancer[11], anticonvulsant[12], anti-HIV[13], antimicrobial[14], antioxidant[15], antitubercular[16], antiviral[17], anti-inflammatory [18] and etc.

1,2,3-Triazoles are an important class of heterocycles due to their wide range of applications as synthetic intermediates and pharmaceuticals[19]. Several therapeutically interesting 1,2,3-triazoles have been reported, including anti-HIV agents[20, 21], antimicrobial

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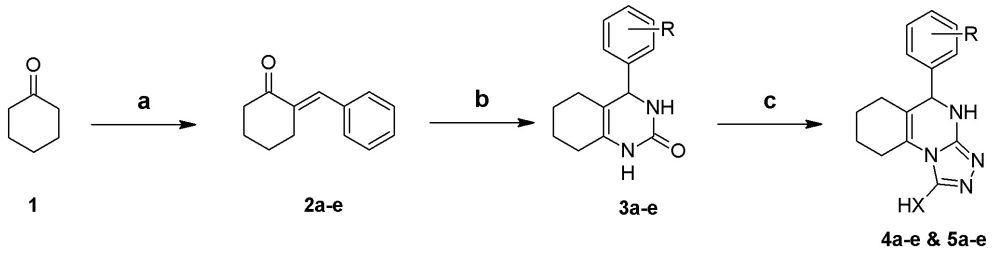

Scheme 1. *Reagents and conditions:* (a) aryl aldehyde, NaOH, EtOH, Strring<10 °C, 2-4h; (b) Urea, KOH, EtOH, reflux, 4-6h; (c) semicarbazide HCl/thiosemicarbazide HCl, KOH, EtOH, reflux, 4-5h.

compounds[22], β3-selective adrenergic receptor agonists[23], kinase inhibitors[24, 25], aceylcholinesterase inhibitors[26], carbonic anhydrase II inhibitors[27] and etc. Along with the diverse biological activities of quinazoline and its derivatives, quinazoline fused with other heterocycles play an essential role in several biological processes and have a considerable chemical and pharmacological importance.

Drug discovery is considerably directed towards identification of new and safe synthetic antiglycating agents and is an important area of pharmaceutical research for the treatment of late diabetic complications[28-31]. Since the availability of an ideal antiglycating agent is less, it instiates the research on molecules for their antiglycating activity[6]. The main part of our research has been devoted to synthetic methods leading to new ring systems, containing the triazolo hexahydroquinazolines nucleus, as a pharmacophore moiety for potential drugs. Hence, *in vitro* antiglycation and antioxidant activities of some 5-aryl-triazolo [4,3-a] hexahydroquinazolines was considered for present investigation.

2. Results and Discussion

2.1. Chemistry

The final compounds **4a-4e & 5a-5e** were synthesized following the synthetic route outlined in **Scheme 1**. All the 2-arylidenecyclohexanone (**2a-e**) were prepared based on Claisen-Schmidt condensation between the aromatic aldehydes and cyclohexanone. Cyclization of **2a-e** with urea in ethanolic KOH provided **3a-e.** Reaction of **3a-e** with semicarbazide HCl and thiosemicarbazide HCl in ethanolic KOH offered **4a-e** and **5a-e** respectively and were characterized by their IR, 1H-NMR and ES-MS spectral and elemental analysis data. CHNS microanalysis revealed that variation in experimental values compared with calculated values is within ±0.4%. All the 5-(4-aryl)-4,5,6,7,8,9-hexahydro-[1,2,4]triazolo[*4,3-a*]quinazolin-1-ol (**4a-4e**) derivatives showed a characteristic O-H stretch (between 3329-3345 cm^{-1}), NH stretch (between 3123-3201 cm^{-1}) and C=N stretch (between 1637-1658 cm^{-1}). In ^{1}H-NMR spectrum displayed a characteristic peak for triazolo hydroxy proton (-OH) between δ7.63-8.10 ppm as singlet, hexahydroquinazoline NH proton between δ 5.50-5.72 ppm as singlet, CH proton between δ 4.60-4.81 ppm as singlet and cyclohexyl-CH_2 protons displayed a triplet of multiplet between δ 1.50-3.38 ppm. All the 5-(4-aryl)-4,5,6,7,8,9-hexahydro-[1,2,4]triazolo[*4,3-a*]quinazolin-1-thiol (**5a-5e**) derivatives showed a characteristic NH stretch (between 3145-3234 cm^{-1}), S-H stretch (between 2532-2554 cm^{-1}) and C=N stretch (between 1555-1642 cm^{-1}). In ^{1}H-NMR spectrum displayed a characteristic peak for triazolo thiol proton (-SH) between δ7.96-8.14 ppm as singlet, CH proton between δ 4.37- 4.70 ppm as singlet, hexahydroquinazoline NH proton between δ 3.90-4.25 ppm as singlet and cyclohexyl-CH_2 protons displayed a triplet of multiplet between δ 1.24-2.80 ppm. The EI-MS spectra of all the compounds (**4a-4e** & **5a-5e**) displayed $(M+1)^+$ peak. The structure and physical characterization of compounds **4a-e** and **5a-e** is presented in **Table 1.**

Table 1. Physical Properties of 4a-e & 5a-e

Code	R	X	MF	MW	MP(°C)
4a	phenyl	O	$C_{15}H_{16}N_4O$	268	220-222
4b	*p*-OCH_3 phenyl	O	$C_{16}H_{18}N_4O_2$	298	186-188
4c	*p*-Cl phenyl	O	$C_{15}H_{15}ClN_4O$	302	196-198
4d	furan-2-yl	O	$C_{13}H_{14}N_4O_2$	258	230-232
4e	*p*-NO_2 phenyl	O	$C_{15}H_{15}N_5O_3$	313	215-217
5a	phenyl	S	$C_{15}H_{16}N_4S$	284	184-186
5b	*p*-OCH_3 phenyl	S	$C_{16}H_{18}N_4O_S$	314	210-214
5c	*p*-Cl phenyl	S	$C_{15}H_{15}ClN_4S$	318	164-168
5d	furan-2-yl	S	$C_{13}H_{14}N_4OS$	274	221-223
5e	*p*-NO_2 phenyl	S	$C_{15}H_{15}N_5O_2S$	329	198-200

2.2. Anti glycating activity

All the synthesized **4a-4e & 5a-5e** were randomly evaluated for their *in vitro* antiglycating activity. The compounds exhibited a varying degree of antiglycating activity, when compared to standards rutin (antiglycating agent; IC_{50} = 41.9 μM) and glibenclamide (antidiabetic agent; 0.002 μM) **(Table 1).** Comparatively, all the synthesized compounds are potent than rutin in the range between 0.096 and 0.300 μM. The phenyl ring substituted with electron donating group have shown more potency than electron withdrawing substituent i.e. **4b** (*p*-OCH_3 phenyl) and **5c** (*p*-Cl phenyl), also excellent antiglycation activities, when compared to standard rutin, but less potent than glibenclamide (IC_{50} = 0.002 μM). Additionally, compounds **4e** (*p*-NO_2 phenyl) and **5b** (*p*-OCH_3) initially showed less than 50 % activity and thus were not further evaluated for their IC_{50}. The thiol derivatives are more potent than the corresponding hydroxyl derivatives, as **5a (**IC_{50} = 0.117μM) is more potent than **4a (**IC_{50} = 0.191 μM) and **5c (**IC_{50} = 0.096 μM) is more potent than **4c (**IC_{50} =0.300μM)**.** Compound **4b, 4d, 5a, 5c,** and **5e**have shown more potent anti-glycating activity than all other derivatives. Among these compounds **4b** and **5c** are more potent. Within this small set of active molecules, a concrete SAR discussion may not be feasible. But few guiding principles can be derived to proceed further in designing this class of molecules as antiglycating agents. The IC_{50} values of anti-glycating activity given in **Table 2.**

Table 2. IC_{50} values for antiglycating activity of 4a-e & 5a-e

Code	R	X	IC50 (μM)
4a	phenyl	O	0.191
4b	p-OCH3 phenyl	O	0.104
4c	p-Cl phenyl	O	0.300
4d	furan-2-yl	O	0.134
4e	p-NO2 phenyl	O	ND
5a	phenyl	S	0.117
5b	p-OCH3 phenyl	S	ND
5c	p-Cl phenyl	S	0.096
5d	furan-2-yl	S	0.192
5e	p-NO2 phenyl	S	0.102
RTN	-	-	41.9
GBD	-	-	0.002

ND: Not Determined; RTN-Rutin; GBD-Glibenclamide

Antioxidant activity:

All the synthesized compounds **(4a-4e& 5a-5e)** were tested for antioxidant activity by DPPH assay. These compoundsexhibited potent to moderate antioxidant activity in the present investigation. In the series, almost all the test compounds were found to be potent than BHT (Butylated Hydroxytoluene) except **5e** (*p*-NO_2 phenyl). Among all,the hydroxyl derivatives (**4a-4e)** are more potent than the corresponding thiols (**5a-5e**). Molecules **4c** (p-Cl phenyl) and **4b** (p-OCH_3) were the most potent compounds. The IC_{50} values were shown in **Table 3.**

Table 3. IC_{50} values for antioxidant activity of 4a-e & 5a-e

Code	R	X	IC_{50} (μM)
4a	phenyl	O	0.213
4b	*p*-OCH_3 phenyl	O	0.122
4c	*p*-Cl phenyl	O	0.093
4d	furan-2-yl	O	0.237
4e	*p*-NO_2 phenyl	O	0.122
5a	phenyl	S	0.137
5b	*p*-OCH_3 phenyl	S	0.226
5c	*p*-Cl phenyl	S	0.269
5d	furan-2-yl	S	0.216
5e	*p*-NO_2 phenyl	S	0.589
BHT	-		0.510

BHT-Butylated hydroxy toluene

3. Experimental

Materials and methods: All the reagents and chemicals were obtained commercially from Merck, Spectrochem or CDH, India. Purified solvents of reagent grade were purified, dried by standard procedure. Monitoring of reactions and homogeneity of the intermediate and final compounds were carried out by thin-layer chromatography on silica gel plates and visualized in either iodine or UV chambers. Melting points were determined using Thermonik Melting Point Apparatus (Campbell electronics, India) by open capillary method and are uncorrected. Infrared (IR) spectra were taken on a FT-IR Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000-400 cm^{-1} using KBr discs. ^{1}H-NMR spectra were recorded at 400 MHz in DMSO-d_6 using a BrukerAvance 400 instrument (Bruker Instruments Inc., USA). Chemical shifts were measured at δ units (ppm) relative to Tetramethylsilane (TMS). Fast-atom bombardment (FAB) mass spectra were recorded on a Jeol SX 102/DA-6000 mass spectrometer (Jeol Ltd. Akishima, Tokyo, Japan) using argon/xenon (6kV, 10mA) as FAB gas, m-nitrobenzyl alcohol as matrix, and 10 kV as accelerating voltage at room temperature. Elemental analysis (%C, H, N) was performed on a Vario EL III Elemental Analyser (Elementar, Germany). The 5-aryl-triazolo [4,3-a]hexahydroquinazolines were synthesized as per **Scheme 1** and characterized by elemental analysis, IR, ^{1}H-NMR and EI-MS. In the elemental analysis, the observed values were within ±0.4% of the calculated values.

3.1. Chemistry

*3.1.1. General procedure for synthesis of 2-arylidenecyclohexanone (**2a-2e**)*

Cyclohexanone (0.01 mole), ethanolic NaOH (20 %; 20ml) were taken in a round bottom flask and drop wise addition of respective benzaldehyde (0.01 mole) with continuous stirring for 2 h. Further stirred was continued for 1 hr and mixture was kept in the refrigerator for overnight. The obtained yellowish precipitate was collected by filtration and crude product was recrystallized with chloroform.

3.1.2. General procedure for synthesis of 4-aryl-3,4,5,6,7,8-hexahydroquinazolin-2(1H)-one (**3a-3e**):

The 2-arylidenecyclohexanone (**2**, 0.01 mol), urea (0.02 mol) were taken in ethanolic KOH (20 %; 20 ml) and heated to reflux for 4-6 h. Completion of reaction was monitored by TLC and excess of solvent was removed *in vacuo*. To the residue, cold water (25 ml) was added and neutralized with dil. HCl, then kept in refrigerator ice-chest for overnight. Collected the crystalline product of **3** by vacuum filtration and further purified by recrystallization with chloroform.

*3.1.3. General procedure for synthesis of 5-(4-aryl)-4,5,6,7,8,9-hexahydro-[1,2,4]triazolo[4,3-a]quinazolin-1-ol (**4a-4e**):*

A mixture of 4-aryl-3,4,5,6,7,8-hexahydroquinazolin-2(1H)-ones (**3,** 0.005 M) and semicarbazide HCl (0.01 M) were dissolved in ethanolic KOH (10 %; 20 ml) in a round bottom flask. The reaction mixture was heated to reflux for 4-5 hrs and progress of reaction was monitored by TLC. After completion of reaction, the mixture was cooled to room temperature then poured into beaker containing ice cold water and neutralized with the addition dil HCl. Precipitate obtained was filtered and dried. Spectroscopic and elemental analysis data of the synthesized compounds **4a-4e** given below:

*3.1.4. General procedure for synthesis of 5-(4-aryl)-4,5,6,7,8,9-hexahydro-[1,2,4]triazolo[4,3-a]-quinazolin-1-thiol (**5a-5e**):*

A mixture of 4-aryl-3,4,5,6,7,8-hexahydroquinazolin-2(1H)-ones(**3,** 0.005 M) and thiosemicarbazide HCl (0.01 M) were dissolved in ethanolic KOH (10 %; 20 ml) in a round bottom flask. The reaction mixture was heated to reflux for 4-5 hrs and progress of reaction was monitored by TLC. After completion of reaction, the mixture was cooled to room temperature then poured into beaker containing ice cold water and neutralized with the addition of dil HCl. Precipitate obtained was filtered and dried.Spectroscopic and elemental analysis data of the synthesized compounds **5a-5e** given below:

*5-phenyl-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3-a]quinazo lin-1-ol **(4a)**:* IR (KBr, cm^{-1}): 3329 (O-H stretch), 3123 (NH stretch), 1652 (C=N stretch); ^{1}H-NMR (DMSO-d6, δ ppm): 7.90 (s, 1H, OH), 6.60-7.40 (m,

5H, Ar.H), 4.78 (s, 1H, 1NH), 3.75 (s, 1H, CH), 2.25-2.38 (m, 4H, cyclohexyl-CH_2), 1.88-1.96 (t, 4H, cyclohexyl-CH_2); Anal. Calcd. for $C_{15}H_{16}N_4O$ (268.31): C, 67.15; H, 6.01; N, 20.88%. Found: C, 67.28; H, 5.90; N, 20.92%; MS *m/z*: 269 $(M+1)^+$

5-(4-methoxyphenyl)-4,5,6,7,8,9-hexahydro- [1,2,4] triazolo [4,3-a]quinazolin-1-ol ***(4b)****:* IR (KBr, cm^{-1}): 3334 (O-H stretch), 3128 (NH stretch), 1644 (C=N stretch); ^{1}H-NMR (DMSO-d_6, δ ppm): 8.10 (s, 1H, OH), 6.41-7.11 (m, 4H, Ar.H), 5.60 (s, 3H, OCH_3), 4.81 (s, 1H, 1NH), 3.90 (s, 1H, CH), 1.50-3.20 (m, 8H, cyclohexyl-CH_2); Anal. Calcd. for $C_{16}H_{18}N_4O_2$ (298.34): C, 64.41; H, 6.08; N, 18.78%. Found: C, 64.58; H, 6.09; N, 18.84%; MS *m/z*: 299 $(M+1)^+$

5-(4-chlorophenyl)-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3 -a]quinazolin-1-ol ***(4c)****:* IR (KBr, cm^{-1}): 3330 (O-H stretch), 3201 (NH stretch), 1640 (C=N stretch); ^{1}H-NMR (DMSO-d6, δ ppm): 7.90 (s, 1H, OH), 7.43 (dd, 2H, Ar.H), 7.35 (dd, 2H, Ar.H), 4.67 (s, 1H, 1NH), 3.80 (s, 1H, CH), 2.25-2.38 (m, 4H, cyclohexyl-CH_2), 1.88-1.96 (t, 4H, cyclohexyl-CH_2); Anal. Calcd.for $C_{15}H_{15}ClN_4O$ (302): C, 59.51; H, 4.99; N, 18.51%. Found: C, 59.72; H, 5.00; N, 18.56%; MS *m/z*: 303 $(M+1)^+$

5-(furan-2-yl)-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3-a]quinazolin-1-ol ***(4d)****:* IR (KBr, cm^{-1}): 3338 (O-H stretch), 3143 (NH stretch), 1658 (C=N stretch); ^{1}H-NMR (DMSO-d6, δ ppm): 8.10 (s, 1H, OH), 7.80 (d, 1H, Ar.H), 6.52-7.12 (m, 2H, ArH), 4.76 (s, 1H, 1NH), 3.75 (s, 1H, CH), 1.64-3.38 (m, 8H, cyclohexyl-CH_2); Anal. Calcd. for $C_{13}H_{14}N_4O_2$ (258.27): C, 60.45; H, 5.46; N, 21.69%. Found: C, 60.64; H, 5.45; N, 21.72%; MS *m/z*: 259 $(M+1)^+$

5-(4-nitrophenyl)-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3-a]quinazolin-1-ol ***(4e)****:* IR (KBr, cm^{-1}): 3345 (O-H stretch), 3168 (NH stretch), 1637 (C=N stretch); ^{1}H-NMR (DMSO-d_6, δ ppm): 7.63 (s, 1H, OH), 7.36-7.43 (m, 4H, ArH), 4.60 (s, 1H, 1NH), 3.70 (s, 1H, CH), 2.58-2.64 (m, 4H, cyclohexyl-CH_2), 1.86-1.89 (t, 4H, cyclohexyl-CH_2); Anal. Calcd.for $C_{15}H_{15}N_5O_3$ (313.31): C, 57.50; H, 4.83; N, 22.35%. Found: C, 57.48; H, 4.82; N, 22.42%; MS *m/z*: 314 $(M+1)^+$

5-phenyl-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3-a]quinazoline-1-thiol ***(5a)****:* IR (KBr, cm^{-1}): 3221 (NH stretch), 2547 (S-H stretch), 1575 (C=N stretch); ^{1}H-NMR (DMSO-d6, δ ppm): 8.02 (s, 1H, SH), 6.82-7.80 (m, 5H, Ar-H), 4.68 (s, 1H, 1NH), 3.75 (s, 1H, CH), 2.00-2.60 (m, 4H, cyclohexyl-CH_2), 1.64-1.84 (t, 4H, cyclohexyl-CH_2); Anal. Calcd.for $C_{15}H_{16}N_4S$ (284.37): C, 63.35; H, 5.67; N, 19.70%. Found: C, 63.42; H, 5.68; N, 19.74%; MS *m/z*: 285 $(M+1)^+$

5-(4-methoxyphenyl)-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3-a]quinazoline-1-thiol ***(5b)****:* IR (KBr, cm^{-1}): 3234 (NH stretch), 2554 (S-H stretch), 1560 (C=N stretch); ^{1}H-NMR (DMSO-d6, δ ppm): 7.96 (s, 1H, SH), 6.76-7.82 (m, 4H, Ar.H), 5.65 (s, 3H, OCH_3), 4.37(s, 1H, 1NH), 3.80 (s, 1H, CH), 2.00-2.60 (m, 4H, cyclohexyl-CH_2), 1.64-1.84 (t, 4H, cyclohexyl-CH_2); Anal. Calcd.for $C_{16}H_{18}N_4OS$ (314.41): C, 61.12; H, 5.77; N, 17.82%. Found: C, 61.26; H, 5.78; N, 17.76%; MS *m/z*: 315 $(M+1)^+$

5-(4-chlorophenyl)-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3-a]quinazoline-1-thiol ***(5c)****:* IR (KBr, cm^{-1}): 3216 (NH stretch), 2543 (S-H stretch), 1555 (C=N stretch); ^{1}H-NMR (DMSO-d6, δ ppm): 8.12 (s, 1H, SH), 7.12-7.78 (m, 4H, Ar.H), 4.41(s, 1H, 1NH), 3.90 (s, 1H, CH), 1.56-2.80 (m, 8H, cyclohexyl-CH_2); Anal. Calcd.for$C_{15}H_{15}ClN_4S$ (318): C, 56.51; H, 4.74; N, 17.57%. Found: C, 56.48; H, 4.75; N, 17.54%;MS *m/z*: 319 $(M+1)^+$

5-(furan-2-yl)-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3-a]quinazoline-1-thiol ***(5d)****:* IR (KBr, cm^{-1}): 3145 (NH stretch), 2532 (S-H stretch), 1642 (C=N stretch); ^{1}H-NMR (DMSO-d6, δ ppm): 8.14 (s, 1H, SH), 7.80 (d, 1H, Ar.H), 6.58-7.10 (m, 2H, Ar.H), 4.70 (s, 1H, 1NH), 3.90 (s, 1H, CH), 1.42-1.64 (m, 4H, cyclohexyl-CH_2), 1.24-1.36 (m, 4H, cyclohexyl-CH_2); Anal. Calcd.for $C_{13}H_{14}N_4OS$ (274.34): C, 56.91; H, 5.14; N, 20.42%. Found: C, 57.10; H, 5.15; N, 20.44%; MS *m/z*: 275 $(M+1)^+$

5-(4-nitrophenyl)-4,5,6,7,8,9-hexahydro[1,2,4]triazolo[4,3-a]quinazoline-1-thiol ***(5e)****:* IR (KBr, cm^{-1}): 3226 (NH stretch), 2550 (S-H stretch), 1576 (C=N stretch); ^{1}H-NMR (DMSO-d6, δ ppm): 8.09 (s, 1H, SH), 7.26-7.89 (m, 4H, Ar.H), 4.40 (s, 1H, 1NH), 4.20 (s, 1H, CH), 1.6-1.9 (t, 4H, $2CH_2$) 1.20-1.40 (m, 4H, $2CH_2$) ; Anal. Calcd.for $C_{15}H_{15}N_5O_2S$ (329.37): C, 54.70; H, 4.59; N, 21.26%. Found: C, 54.88; H, 4.60; N, 21.30%; MS *m/z*: 330 $(M+1)^+$

3.2. Anti-glycating activity

In all experiments, the final reaction volume was 60 µL and the reactions were performed in 1.5 ml microcentrifuge tubes. Each tube contains, BSA (20 µL; 10 mg/mL), glucose anhydrous (20 µL; 50 mg/mL) and test sample (20 µL) then final volume maede upto 60 µL with DMSO. Glycated control contains 20 µL BSA, 20 µL glucose and 20 µL sodium phosphate buffer, while blank control contains 20 µL BSA and 40µL sodium phosphate buffer. Glibenclamide was used as the standard. Reaction mixture was incubated at 37 °C for seven days. After incubation, 6 µL (100%) of TCA was added in each tube and centrifuged (15,000 rpm) for four minutes at 4 °C. After centrifugation, the obtained pellets were rewashed with 60 µL (10%) of TCA. The supernatant containing glucose, inhibitor and interfering substance was removed and pellet containing AGE-BSA was dissolved in 60 µL PBS. Assessment of fluorescence spectrum (ex. 370 nm) and change in fluorescence intensity (ex. 370 nm to em. 440 nm) based on AGEs were monitored by using a spectrofluorimeter (RF-1500). The % Inhibition was calculated through the following formula[32] and IC_{50} values were given in **Table 1**:

$$\% \text{ Inhibition} = 1 - \frac{\text{Fluorescence of sample}}{\text{Fluorescence of glycalated sample}} \times 100$$

3.3. Antioxidant activity

The antioxidant activity of the synthesized compounds (**3a-3e & 4a-4e)**and the standard was assessed on the basis of the radical scavenging effect of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH)-free radical activity by modified method[33]. The solutions of the test were prepared in methanol. BHT was used as standard. 0.135 mM DPPH was prepared in methanol and 1 ml of prepared solution of DPPH mixed with 3 ml of methanolic solution of test solutions (20-100 µg/ml) and BHT (Butylated Hydroxytoluene) solution separately. These mixtures were vortexed thoroughly and kept in dark at room temperature for 30 min. the absorbance was measured at 517 nm using Schimadzu UV-1800

Spectrophotometer. Methanol (1 ml) with DPPH solution (1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below[34]:

$$\% \text{ Inhibition of DPPH activity} = \frac{[A_0 - A_s]}{A_0} \times 100$$

Where A_0 is the absorbance of control and A_s is the absorbance of sample.

4. Conclusion

Advanced glycation end products are the main cause for diabetic complications. Aminoguanidine is the prototype molecule for use in the treatment of these complications like retinopathy. In the present study, triazolo- hexahydroquinazolines were synthesized as isosteric modification of the aminoguanidines and evaluated them for antiglycating and antioxidant activities. The molecules **4b** and **5c** were found to have potent antiglycating activity than rutin and less potent than glibenclamide due to the presence of electron donating groups substituted on the benzene ring. The molecules **4b** and **4c** were found to have potent antioxidant activity due to the presence of hydroxyl groups. The results obtained may contribute to the development of an ideal antiglycating agents.

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