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Synthesis, antioxidant and carbonic anhydrase inhibitory potential of Schiff bases of thiazoles

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ABSTRACT

A series of Schiff bases of thiazole **2-29** was synthesized and screened for their possible antioxidant and carbonic anhydrase inhibitory potential. The structures of synthetic compounds were characterized by using spectroscopic techniques including IR, ^{1}HNMR and EIMS. All the synthetic compounds gave satisfactory elemental analysis.

KEY WORDS: Thiazole, Antioxidants, Carbonic anhydrase activities

INTRODUCTION:

Carbonic anhydrase (CA; carbonate hydrolyase, EC 4.2.1.1) is a zinc containing metallo-enzyme found in all phyla of life and intricately involved in many physiological processes. It catalyzes the reversible hydration/dehydration of carbon dioxide/bicarbonate. The recent development of carbonic anhydrase inhibitor is applied for the treatment of glaucoma. Dorzolamide is a sulfonamide, introduced in the market in 1995 by well-known pharmaceutical company Merck. Dorzolamide marketed as its hydrochloride and used for lowering high intraocular pressure in open-angle glaucoma and ocular hypertension. This discovery has renewed the pharmacological interest in this zinc-containing enzyme catalyzing the reversible hydration of carbon dioxide [1].

The zinc metallo-enzyme human carbonic anhydrase II (HCA II) is the most studied and probably the best understood. In humans, carbonic anhydrase exists as a number of isoenzymes, the most active carbonic anhydrase II (CA-II), found primarily in red blood cells (RBCs). Deficiency of carbonic anhydrase II is the primary defect in the syndrome of osteoporosis, renal tubular acidosis, and cerebral calcification. Inhibition of carbonic anhydrase in the ciliary processes of the eye decreases aqueous humor secretion, presumably by slowing the formation of bicarbonate ions with subsequent reduction in sodium and fluid transport. The result is a reduction in intraocular pressure (IOP). Acetazolamide is another example of a carbonic anhydrase inhibitor most often administered, not for its diuretic property, but as treatment for epilepsy, intracranial hypertension, and altitude sickness [2]. Antioxidants are compounds that protect cells from the damage caused by oxidation. Anti means "against," and antioxidants work against, or prevent oxidation. The process by which the body breaks down and builds up molecules is called metabolism. During metabolism, atoms may lose electrons. This loss of electrons is called oxidation, because it is fueled by oxygen.

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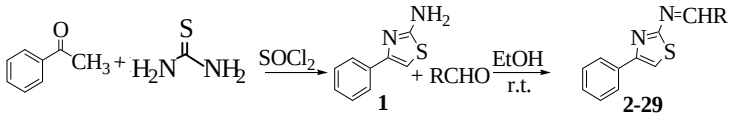
Antioxidants neutralized free radicals before they do harm to our bodies. Free radicals are atoms that cause damage to our cells. They harm our immune system leading to many degenerative diseases. Free radicals are formed from various metabolic processes when our immune systems fight infections. Additional factors that cause free-radical formation include exposure to pollution, excessive sunlight, toxic substances, radiation, tobacco smoke, and asbestos. Continual exposure to these factors leads to uncontrollable free-radical formation and increases the individual's risk for chronic diseases [3]. Antioxidant defense in biological systems has been a growing topic in biomedical sciences. Citrus fruits and juices are important sources of bioactive compounds including antioxidants such as ascorbic acid, flavonoids and phenolic compounds that are important to human nutrition [4].

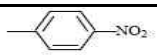
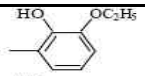
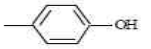
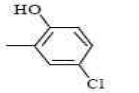
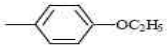
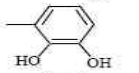
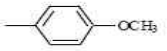
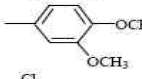
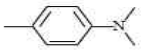
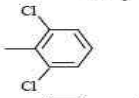
One of the most significant sites of free-radical damage is the cell membrane. Free radicals that form within the phospholipid's bilayer of cell membranes steal electrons from their stable lipid molecules. When the lipid molecules, which are hydrophobic, are destroyed, they no longer repel water. With the cell membrane's integrity lost, the ability to regulate the movement of fluids and nutrients in and out of the cell is also lost. This loss of cell integrity causes damage to the cell and all systems may get affected by this cell.

In cells, Fe^{2+} and Cu^{+} react with hydrogen peroxide to form hydroxyl radical, a highly reactive species that damages DNA. This DNA damage is an underlying cause of neurodegenerative and cardiovascular diseases, as well as many cancers. Antioxidants prevent hydroxyl radical from damaging DNA, and are of interest to treat and prevent these diseases. Numerous diseases are coupled with free-radical production, including: various cancers, heart diseases, diabetes, arthritis, cataracts, kidney diseases, Alzheimer disease, and Parkinson disease [5].

Thiazoles are one of the most important classes of heterocyclic compounds and known for their broad spectrum of biological activities [6]. Many natural and synthetic molecules containing the thiazole moiety play a significant role in the pharmaceutical industry due to their anti-inflammatory [7,8], anti-HIV [9], anti-bacterial [10], anti-cancer [11] properties. These have also found applications in drug development for the treatment of mental retardation in children, age related and neurodegenerative brain damage (Alzheimeris disease, Parkinsonism disease) [12]. Thiazole natural products, such as the mycothiazole, and cystothiazole are a diverse and biologically significant class of compounds in which there has been considerable interest. Due to biological and pharmaceuticals importance of thiazole ring, there has been intense development of methodology to synthesize these moieties [13].

The purpose of this study is to develop a new type of Thiazole derivatives containing precursors for carbonic anhydrase inhibitor and antioxidant moieties, and it was expected to find out more potent anti carbonic anhydrase and antioxidant agents with multitarget mechanism from these derivatives. Thiazole derivatives were synthesized by coupling thiazole amine and aldehydes with aromatic moieties to make lead molecules for hybrid drugs for carbonic anhydrase and antioxidant activities. In this study, we have examined the antioxidant and carbonic anhydrase activities of these derivatives. Furthermore, we have also preliminarily studied the structure-activity relationship (SAR) of these kinds of hybrids to attempt to seek a better paradigm for the multi-target-directed drug design strategy [15-19].



Compound No.	R	Compound No.	R
2		16	
3		17	
4		18	
5		19	
6		20	
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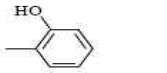
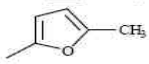
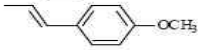
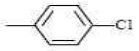
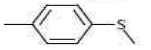
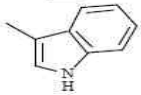
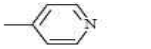
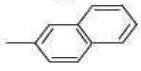
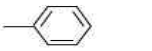
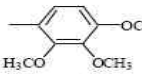
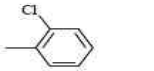
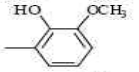
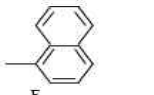
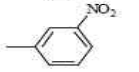
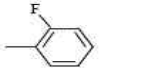
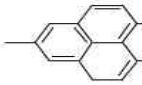
8		22	
9		23	
10		24	
11		25	
12		26	
13		27	
14		28	
15		29	

Scheme-1

EXPERIMENTAL:

Procedure for the synthesis of 4-phenyl-1,3-thiazol-2-amine (1)

To a mixture of acetophenone (0.2 mol) and thiourea (0.4 mol), thionyl chloride (0.2 mol) was added and the reaction mixture was heated on a steam bath for 24 h. The progress of reaction was monitored by TLC, after the completion of reaction the crude product was washed by hexane and crystallized with ethanol.

General procedure for the synthesis of thiazole Schiff bases (2-29).

A mixture of 2-amino-4-phenyl thiazole (0.52 g, 3 mmol) and appropriate aldehydes (3 mmol) in ethanol (15 mL) was stirred at room temperature. The progress of reaction was monitored by TLC, after the completion of reaction solvent was removed on a rotary evaporator, the resulting product was crystallized by appropriate ethanol Scheme-1.

Structure-Activity Relationship of Carbonic Anhydrase Inhibitory Studies:

Compounds **2-29** were screened for their carbonic anhydrase inhibitory potential according to the literature protocol [14]. Fifteen compounds **2, 3, 5, 10, 13, 19, 16, 17, 20, 21, 23, 24, 25, 28,** and **29** possessing IC_{50} values 72.51 ± 0.67, 188.79 ± 2.92, 82.25 ± 0.72, 226.62 ± 3.04, 370.56 ± 8.71, 94.70 ± 7.38, 217.23 ± 2.87, 113.48 ± 8.30, 90.58 ± 2.02, 170.16 ± 2.47, 217.18 ± 5.74, 435.46 ± 9.9, 146.86 ± 4.33, 69.49 ± 4.07, 197.6 ± 2.10 μM, respectively, found to be weak carbonic anhydrase inhibitors if compare with standard acetazolamide (IC_{50} = 0.12 ± 0.03 μM) remaining thirteen compounds **4, 6, 7, 8, 9, 11, 12, 15, 18, 19, 22, 26, 27** were found to be completely inactive Table-1.

Limited SAR study suggests that the activity of the compounds largely depends upon the aromatic substitution. Compound **28** containing nitro group at *meta* position inhibited the enzyme with an IC_{50} value 69.49 ± 4.07 while its *para* analog **2** showed an IC_{50} value 72.51 ± 0.67 μM confirming that *meta* nitro substitution on phenyl ring may be responsible for carbonic anhydrase inhibitory activity in this type of molecules. If we consider all *para* substituted compounds **3, 5, 10, 21, 23** exhibiting IC_{50} values 188.79 ± 2.92, 82.25 ± 0.72, 226.62 ± 3.04, 170.16 ± 2.47 and 217.18 ± 5.74 μM, respectively, Compound **17** possessing IC_{50} value 113.48 ± 8.30, with hydroxyl at *ortho* and chloro at *meta* position showed inhibitory potential may be due to presence of chloro substitution which may increase the charge density around the molecule. Evaluation of activity between **21** and **23** which are isopropyl and chloro substituted, respectively, having IC_{50} values 170.16 ± 2.47 and 217.18 ± 5.74 μM suggested that compound **21** was more active than compound **23** due to the long chain as compared to chloro substitution. Compound **24** with IC_{50} value 435.46 ± 9.9 μM showed activity due to the presence of five membered heterocyclic indole ring.

If we compare the activity of compounds **13** and **20** which are 2-chloro and 2,6-dichloro exhibiting IC_{50} values 370.56 ± 8.71 and 90.58 ± 2.02 μM clearly demonstrate that the activity of compound **20** in comparison to compound **13** may be due to the disubstituted halogen. If we put side by side compounds **14, 25** and **29** having fused benzene ring, then we can clearly see that the compounds **14** and **25** were more active than **29** due to the fusion of two phenyl ring. Compound **16** with IC_{50} value 217.23 ± 2.87 μM was also an inhibitor of this enzyme with the substitution of hydroxyl at *ortho* and ethoxy at *meta* position may be the reason of inhibition enzyme.

Table-1: Carbonic anhydrase inhibitory activities of thiazole Schiff bases 2-29

Compounds	IC_{50} ± SEM[a] μM	Compounds	IC_{50} ± SEM[a] μM	Compounds	IC_{50} ± SEM[a] μM
2	72.51 ± 0.67	**12**	NA[b]	**22**	NA[b]
3	188.79 ± 2.92	**13**	370.56 ± 8.71	**23**	217.18 ± 5.74
4	NA[b]	**14**	94.70 ± 7.38	**24**	435.46 ± 9.9
5	82.25 ± 0.72	**15**	NA[b]	**25**	146.86 ± 4.33
6	NA[b]	**16**	217.23 ± 2.87	**26**	NA[b]
7	NA[b]	**17**	113.48 ± 8.30	**27**	NA[b]
8	NA[b]	**18**	NA[b]	**28**	69.49 ± 4.07
9	NA[b]	**19**	NA[b]	**29**	197.6 ± 2.10
10	226.62 ± 3.04	**20**	90.58 ± 2.02	**Acetazolamide[c]**	0.12 ± 0.03
11	NA[b]	**21**	170.16 ± 2.47		

[a]SEM is the standard error of the mean, NA[b] Not Active, Acetazolamide[c] Standard inhibitor for carbonic anhydrase inhibitory activity

Structure-Activity Relationship of Antioxidant Assay

Twenty eight synthesized Schiff bases were subjected for their antioxidant assays according to literature protocol [15]. Thiazoles derivatives **2-29** demonstrated varying degree of free radical scavenging activity when compared to standard ascorbic acid (IC_{50} = 9.37 *m*M) as shown in Table- 2. Compound **6-7, 13, 18-20, 23-24, 28** and **29** having same value of EC_{50} = 37.5 were found to be moderately active. Compounds **3-4, 9-11, 14-15, 21, 26** and **27** were the weak inhibitors of the series. The compounds **8** and **22** showed less than 50% of inhibition, so these were not further evaluated and considered to be inactive.

Table-2: Antioxidant inhibitory activities of thiazole Schiff bases 2-29

Compound	EC_{50}	Compound	EC_{50}	Compound	EC_{50}
2	18.75	**12**	18.75	**22**	NA[b]
3	150	**13**	37.5	**23**	37.5
4	150	**14**	75	**24**	37.5
5	18.75	**15**	150	**25**	18.75
6	37.5	**16**	18.75	**26**	75
7	37.5	**17**	18.75	**27**	150
8	NA[b]	**18**	37.5	**28**	37.5
9	75	**19**	37.5	**29**	37.5
10	75	**20**	37.5	**Ascorbic acid[c]**	9.37
11	150	**21**	150		

MATERIAL AND METHODS:

***In vitro* Carbonic Anhydrase Assay Protocol:**

4-NPA, which is colorless, in this assay, on hydrolysis converted to 4-nitrophenol and carbondioxide and which is followed by measuring the formation of 4-nitrophenol, a yellow colored compound. The reaction was carried out at 25-28 °C. The experiment was carried out in buffer solution having HEPES an acidic and *tris* alkaline at a total concentration of 20 *m*M and pH ranges from 7.2-7.9. For every test compound the reaction tube consisted of 140 µL of the HEPES-*Tris* solution, 20 µL of newly prepared aqueous solution of purified bovine erythrocyte CA-II (0.1-0.2mg/2000µL of deionized water for 96-well plates), sigma Aldrich. 20 µL of test compound which was dissolved in DMSO and 20 µL of substrate 4-NPA at concentration of 0.7 *m*M diluted in ethanol [14]. The experiment was carried out in triplicates.

Antioxidant (DPPH) (1,1-Diphenyl-2-Picryl Hydrazyl) Free Radical Scavenging Activity

The free radical scavenging activity was measured by 1,1-diphenyl-2-picrylhydrazil (DPPH) using literature protocols [15]. Reaction mixture contains 5 μL of test sample (1 *m*M in DMSO) and 95 μL of DPPH (Sigma, 300 μM) in ethanol. The reaction mixture was taken into a 96-well microtiter plate and incubated at 37 °C for 30 min. The absorbance was measured at 515 nm on microliter plate reader (Molecular Devices, USA). IC_{50} values represent concentration of compounds to scavenge 50% of DPPH radicals. Ascorbic acid was used as a positive control. All the chemicals used were of analytical grade (Sigma, USA).

REFERENCES

1. S. Lindskog, *Pharmacol. Thu.*, **1997**, *74*, l-20.
2. B. S. Avvaru, S. A. Busby, M. J. Chalmers, P. R. Griffin, B. Venkatakrishnan, M. A. McKenna, D. N. Silverman, R. McKenna, *Biochemistry* **2009**, *48*, 7365-7372.
3. C. Y. Hsu, Y. P. Chan, J. Chang, *Biol Res.*, **2007**, *40*, 13-21.
4. K. Ghasemi, Y. Ghasemi, M. A. Ebrahimzadeh, *Pak. J. Pharm. Sci.*, **2009**, *22*, 277-281.
5. S. M. Phipps, M. H. M. Sharaf, V. Butterweck, *Pharmacop. For.*, **2007**, *33*, 810-814.
6. Y. Izumisawa, H. Togo, *Green. Sustainable Chem.*, **2011**, *1*, 54-62
7. F. Haviv, J. D. Ratajczyk, R. W. DeNet, F. A. Kerdesky, R. L. Walters, S. P. Schmidt, J. H. Holms, P. R. Young and G. W. Carter, *J. Med. Chem.*, 1988, *31*, 1719-1728.
8. F. Clemence, O. L. Marter, F. Delevalle, J. Benzoni, A. Jouanen, S. Jouquey, M. Mouren and R. Deraedt, *J. Med. Chem.*, **1988**, *31*, 1453-1462.
9. F. W. Bell, A. S. Cantrell, M. Hogberg, S. R. Jaskunas, N. G. Johansson, C. L. Jordon, M. D. Kinnick, P. Lind, J. M. Morin Jr, R. Noreen, B. Oberg, J. A. Palkowitz, C. A. Parrish, P. Pranc, C. Sahlberg, R. J. Ternansky, R. T. Va-sileff, L. Vrang, S. J. West, H. Zhang, X. X. Zhou, *J. Med. Chem.*, **1995**, *38*, 4929-4936.
10. K. Tsuji, H. Ishikawa, *Bioorg. Med. Chem. Lett.*, **1994**, *4*, 1661-1606.
11. X. H. Gu, X. Z. Wan, B. Jiang, *Bioorg. Med. Chem. Lett.*, **1999**, *9*, 569-572.
12. S. Chandrasekhar, Ch. Narsimulu, S. S. Sultana, N. R. K. Reddy, *Org. Lett.*, **2002**, *4*, 4399-4403.
13. X. Gao, Y. Pan, M. Lin, L. Chen, Z. Zhan, *Org. Biomol. Chem.*, **2010**, *8*, 3259-3266.
14. O. Arslan, *Biochemistry*, **2001,** *9*, 982-983.
15. V. Bayazit, K. M. Khan, *J. Chem. Soc. Pak.*, **2005**, *27*, 306-313.
16. K. M. Khan, Z. S. Saify, S. Hayat, M. Z. Khan, F. Noor, M. I. Choudhary, Zia-Ullah, S. Perveen, *J. Chem. Soc. Pak.*, **2002**, *24*, 226-231.
17. K. M. Khan, M. Ali, A. Wadood, Zaheer-ul-Haq, M. Khan, M. A. Lodhi, S. Perveen, M. I. Choudhary, and W. Voelter, *J. Molecu. Graph.Mode.*, **2011**, *30*, 153-156.
18. K. M. Khan, U. R. Mughal, Iman Omar and M. I. Choudhary, *Lett. .Drug. Desig. Discov.*, **2008**, *5*, 152-157.
19. K. M. Khan, Z. S. Saify, S. Hayat, M. Z. Khan, F. Noor, M. I. Choudhary, Zia-Ullah and S. Perveen, *J. Chem. Soc. Pak.*, **2002**, *24*, 226-231.
20. A.W. Bauer, W. M. M. Kirby, J. C. Sherris, M. Turk, *Am. J. Clin. Pathol*., **1966**, *45*, 493-496.

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