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Evaluation of novel heterocyclic compounds containing pyrimidine and thiazolidinone rings

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ABSTRACT

A new class of heterocyclic compounds containing pyrimidine and thiazolidinone rings was screened for anti-diabetic, anti-inflammatory and antioxidant activity by alpha-amylase inhibition, inhibition of protein denaturation and hydrogen peroxide radical scavenging activity respectively by using *in vitro* method. Compounds **E_1, E_3, E_6, E_7, E_{11},** and **E_{12}** have shown promising anti-diabetic activity. Compounds **E_2, E_4, E_6, and E_{10}** have shown promising anti-inflammatory activity at 600 µg/ml. concentration. Compounds **E_1, E_6, E_8, E_9 and E_{11}** have shown promising antioxidant activity at 250 µg/ml.

Keywords: Pyrimidine, Thiazolidinone, Antidiabetic, Anti-inflammatory, Antioxidant.

INTRODUCTION

Over the years pyrimidine and thiazolidinones have emerged as an exciting class of five and six member heterocyclic with an amazingly wide range of applications in medicinal chemistry. Thiazolidinone are well known as constitutional units in several agents possessing antimicrobial [1], antituberculosis [2], anti-HIV activities [3], antischistosomal activity [4], antifungal [5], anti-inflammatory [6], antimalarial [7], herbicidal [8], antiviral [9], anti-diabetic [10], and antioxidant [11] activities..

Pyrimidine, being an integral part of DNA and RNA, imparts to diverse pharmacological properties as effective bactericide and fungicide [12, 13]. Certain pyrimidine derivatives were also known to exhibit antimalarial [14], antifilarial [15], antioxidant [16, 17], anti-HIV activities [18], antipyretic [19], anticancer [20], anti-inflammatory and analgesic [21, 22, 23]. All above biological activities of pyrimidine and thiazolidinone derivatives aroused our attention and promoted to screen the compounds for their potential as an anti-diabetic, anti-inflammatory and antioxidant agent.

MATERIALS AND METHODS

Experimental

The synthesized compounds were selected from our reported literature for the various pharmacological activities such as anti-diabetic, anti-inflammatory and antioxidant activity [24].

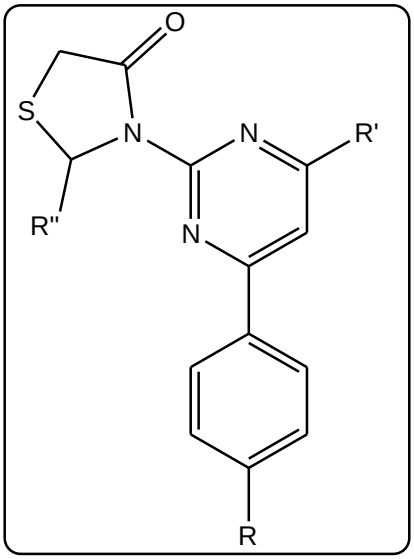

Fig. 1. Novel heterocyclic compounds containing pyrimidine and thiazolidinone rings (E_1-E_{12})

Com. Code	R	R'	R''	Com. Code	R	R'	R''
E_1	$-OCH_3$	$4\text{-}NO_2.C_6H_5$	$4\text{-}OH.C_6H_5$	**E_7**	-OH	$4\text{-}NO_2.C_6H_5$	$4\text{-}Cl.C_6H_5$
E_2	$-OCH_3$	$4\text{-}NO_2.C_6H_5$	$4\text{-}NO_2.C_6H_5$	**E_8**	-OH	$4\text{-}NO_2.C_6H_5$	2-furyl
E_3	$-OCH_3$	$4\text{-}NO_2.C_6H_5$	$4\text{-}Cl.C_6H_5$	**E_9**	$-OCH_3$	$4\text{-}Cl.C_6H_5$	$4\text{-}OH.C_6H_5$
E_4	$-OCH_3$	$4\text{-}NO_2.C_6H_5$	2-furyl	**E_{10}**	$-OCH_3$	$4\text{-}Cl.C_6H_5$	$4\text{-}NO_2.C_6H_5$
E_5	-OH	$4\text{-}NO_2.C_6H_5$	$4\text{-}OH.C_6H_5$	**E_{11}**	$-OCH_3$	$4\text{-}Cl.C_6H_5$	$4\text{-}Cl.C_6H_5$
E_6	-OH	$4\text{-}NO_2.C_6H_5$	$4\text{-}NO_2.C_6H_5$	**E_{12}**	$-OCH_3$	$4\text{-}Cl.C_6H_5$	2-furyl

Anti-diabetic activity [25]

The α-amylase inhibition assay was adapted and modified from *Giancarlo et al.* (2006). The starch solution (0.5% w/v) was obtained by boiling and stirring 0.25 g of potato starch in 50 ml of deionized water for 15 min. The enzyme solution (0.5 unit/mL) was prepared by mixing 0.001 g of α-amylase (EC 3.2.1.1) in 100 ml of 20 mM sodium phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. The compounds were dissolved in DMSO to give concentrations from 20 to 80 mg/ml (20, 40, 60, 80 mg/ml). The color reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (20 ml), 5.31 M sodium potassium tartarate in 2 M sodium hydroxide (8 mL) and deionized water (12 mL). 1 ml of compound solution and 1 mL enzyme solution were mixed in a tube and incubated at 25°C for 30 min. To 1 mL of this mixture 1 mL of starch solution was added and the tube incubated at 25°C for 3 min. Then, 1 mL of the color reagent was added and the closed tube placed into an 85°C water bath. After 15 min, the reaction mixture was removed from the water bath and cooled thereafter, diluted with 9 mL distilled water and the absorbance value determined at 540 nm in a Shimadzu Multispect-1501 spectrophotometer. Individual blanks were prepared for correcting the background absorbance. In this case, the color reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. The other procedures were carried out as above. Controls were conducted in an identical fashion replacing compound sol with 1 mL DMSO. Acarbose solution (at the concentrations of 20, 40, 60, 80 µg/mL) was used as positive control. The inhibition percentage of α-amylase was assessed by the following formula:

$I_{\alpha\text{-amylase}}$ % = 100 × ($\Delta A_{Control} - \Delta A_{Sample}$) / $\Delta A_{Control}$

$\Delta A_{Control} = A_{Test} - A_{Blank}$ and $\Delta A_{Sample} = A_{Test} - A_{Blank}$

The $I_{\alpha\text{-amylase}}$ % was plotted against the sample concentration and a logarithmic regression curve

established in order to calculate the IC_{50} value (inhibitory concentration). This would represent the concentration of sample

Anti- inflammatory activity: Inhibition of Protein Denaturation [26-28]

The standard drug and synthesized compounds were dissolved in minimum quantity of dimethyl formamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all solution was less than 2.5%. Test solution (1 mL) containing different concentrations of drug was mixed with 1 ml of 1 mM albumin solution in phosphate buffer and incubated at 27° + 1°C in BOD incubator for 15 min. Denaturation was induced by keeping the reaction mixture at 60° + 1°C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is taken. The diclofenac sodium was use as standard drug.

The percentage inhibition of denaturation was calculated by using following formula.

% of Inhibition = 100 X [1- Vt / Vc]

Where, Vt = Mean absorbance of test sample and

Vc = Mean absorbance of control

Antioxidant activity: Hydrogen Peroxide Radical Scavenging Activity [29, 30]

1 mL of (20 – 200 µg/mL) test drug/standard (Ascorbic acid) was added to 0.6 mL of hydrogen peroxide solution (Ashwin fine chemicals and pharmaceuticals) in phosphate buffer (P^H - 7.4). After incubating for 10 min. at 37°C the absorbance was measured at 230 nm. Corresponding blanks were taken. The experiment was performed in triplicate. The absorbance of hydrogen peroxide in phosphate buffer as control was measured at 230 nm. The scavenging effect (%) was measured using following equation. Hydrogen peroxide produces hydroxyl radicals in cells. Scavenging of these radicals by the test drug is used as a test for antioxidant activity. The reduction of these radicals is seen by the decreased absorbance at 230 nm with increasing concentration of the test drug.

$$\text{Scavenging effect (\%)} \quad \frac{\text{Control absorbance} - \text{Test absorbance}}{\text{Control absorbance}} \quad X\ 100$$

Compounds **E_1, E_6, E_8, E_9 and E_{11}**have shown promising antioxidant activity at 250 µg/ml, while the remaining compounds have also shown moderate antioxidant activity, when compared with standard drug ascorbic acid.

RESULTS

Anti-Diabetic Activity

All the compounds **(E_1-E_{12})** were screened for their anti-diabetic activity by *In-vitro* alpha-amylaseinhibitionmethod with standard drug acarbose. The compounds **E_1, E_3, E_6, E_7, E_{11}, and E_{12}**have shown significant anti-diabetic activity. Comparing alpha amylase inhibitory effects of various compound, it was observed that compound **E_1** exhibited appreciable alpha amylase inhibitory effects (IC_{50} value 37.5± 2.32 µg/mL) when compared with acarbose (IC_{50} 14.5 ± 4.01 µg/mL).

Table 1. Anti-diabetic activity of the synthesized compounds

Comp Code	20 mg/mL		40 mg/mL		60 mg/mL		80 mg/mL	
	Absorbance	% inhibition	Absorbance	% inhibition	Absorbance	% inhibition	Absorbance	% inhibition
E_1	0.37** ±0.01	41.5	0.31** ±0.018	52	0.23** ±0.046	63	0.18*** ±0.038	73

E_2	0.50* ±0.023	10.4	0.5* ±0.026	9.6	0.501* ±0.025	10.8	0.51* ±0.012	79
E_3	0.44** ±0.011	30.5	0.33** ±0.034	48	0.25** ±0.034	61	0.2** ±0.026	69
E_4	0.63 ns ±0.024	0	0.63 ns ±0.037	0	0.6ns ±0.017	3.1	0.61ns ±0.011	4.1
E_5	0.63 ns ±0.031	0	0.6 ns ±0.041	5	0.63 ns ±0.012	0	0.63 ns ±0.021	0
E_6	0.48** ±0.038	25	0.38** ±0.019	40	0.3** ±0.016	54	0.21** ±0.010	68.8
E_7	0.43** ±0.04	32	0.42** ±0.039	43	0.31** ±0.018	54	0.35** ±0.027	54
E_8	0.61 ns ±0.027	4.3	0.61 ns ±0.043	3.7	0.63 ns ±0.038	0	0.63 ns ±0.015	0
E_9	0.6 ns ±0.038	5	0.60 ns ±0.016	3	0.59 ns ±0.010	7	0.63 ns ±0.037	0
E_{10}	0.51* ±0.013	7	0.63 ns ±0.011	0	0.63 ns ±0.037	0	0.53 * ±0.011	6
E_{11}	0.49** ±0.036	20.3	0.48** ±0.048	27.5	0.4** ±0.035	38	0.34** ±0.047	47
E_{12}	0.5* ±0.041	22	0.43** ±0.036	32	0.37** ±0.018	41	0.28** ±0.019	56
Acarbose	0.3** ±0.018	53	0.24** ±0.015	62	0.19*** ±0.028	71	0.12*** ±0.039	81

One way ANOVA followed by Dunnett's't' test,*P<0.01, **P<0.001, *P<0.0001, ns- non significant.

Compounds **E_1, E_3, E_6, E_7, E_{11}, and E_{12}** have shown promising anti-diabetic activity.

Compound	IC_{50} mg/ml
E1	37.5
E3	43
E6	52
E7	54
E11	87
Acarbose	12.5

Anti-inflammatory activity

In case of *in-vitro* anti-inflammatory activity at different concentration like 200 µg/mL, 400 µg/mL, 600 µg/mL, and 800 µg/mL by inhibition of protein denaturation method. Compounds **E_2, E_4, E_6 and E_{10}** have shown promising anti-inflammatory activity at 600 µg/mL concentration when compared with standard drug diclofenac Sodium.

Table 2 Anti- inflammatory activity of the synthesized compounds

Comp. code	200 µg/mL		400 µg/mL		600 µg/mL		800 µg/mL	
	Absorbance	**% inhibition**	**Absorbance**	**% inhibition**	**Absorbance**	**% inhibition**	**Absorbance**	**% inhibition**
E_1	0.08* ±0.02	51.45	0.06* ±0.03	67.37	0.04* ±0.02	76.55	0.05* ±0.031	72.54
E_2	0.092* ±0.03	46.06	0.071* ±0.022	58.68	**0.038* ±0.03**	**81.39**	0.07* ±0.021	65.96
E_3	0.09** ±0.025	50.36	0.05** ±0.02	74.38	0.06*±0.021	68.06	0.076* ±0.023	55.48
E_4	0.087* ±0.02	48.98	0.07* ±0.02	64.57	**0.036* ±0.028**	**79.39**	0.08* ±0.026	49.65
E_5	0.13ns	8.01	0.16 ns	5.94	0.13 ns	20.04	0.17 ns	6.89
E_6	0.108 ns	37.05	0.079* ±0.03	53.54	**0.023** ±0.031**	**80.61**	0.06* ±0.021	67.32
E_7	0.076* ±0.023	55.37	0.062* ±0.021	69.03	0.062* ±0.034	69.05	0.071* ±0.025	62.06
E_8	0.088* ±0.02	49.23	0.06* ±0.032	67.09	0.04* ±0.024	75.39	0.07* ±0.027	64.17
E_9	0.092* ±0.03	45.95	0.076* ±0.026	55.39	0.05*±0.032	74.67	0.076 ±0.023	55.79
E_{10}	0.075* ±0.024	56.21	0.072* ±0.020	61.05	**0.026** ±0.02**	**82.20**	0.079* ±0.036	53.12
E_{11}	0.16 ns	3.04	0.16 ns	5.35	0.14 ns	21.28	0.13 ns	4.43
E_{12}	0.074* ±0.021	57.00	0.07* ±0.024	63.29	0.07* ±0.03	65.74	0.075* ±0.035	54.68
Diclofinac. Sod.	0.055* ±0.02	68.5	0.03* ±0.021	88.2	**0.02** ±0.03**	**89.2**	0.035* ±0.02	70.3

One way ANOVA followed by Dunnett's't' test , *P<0.05, **P<0.001, ns- non significant

Antioxidant activity

In case of *In-vitro* antioxidant activity at different concentration by hydrogen peroxide radical scavenging activity, compounds **E_1, E_6, E_8, and E_9** have shown promising antioxidant activity at 250 µg/mL, while the remaining compounds have also shown moderate antioxidant activity, when compared with standard drug ascorbic acid.

Table 3 Antioxidant activity of the synthesized compounds

Comp. code	50 µg/mL		150 µg/mL		250 µg/mL	
	Absorbance	**% inhibition**	**Absorbance**	**% inhibition**	**Absorbance**	**% inhibition**
E_1	0.09* ±0.037	24.8	0.08* ±0.028	42.7	**0.041** ±0.012**	**72.1**
E_2	0.093* ±0.04	28.6	0.18ns	27.5	0.048* ±0.016	64.6
E_3	0.10ns	18.4	0.09* ±0.041	39.3	0.07* ±0.021	53.3
E_4	0.096* ±0.041	32.6	0.08* ±0.030	45.7	0.046* ±0.017	63.3
E_5	0.095* ±0.036	22.0	0.07* ±0.022	48.2	0.060* ±0.019	55.3
E_6	0.086* ±0.032	15.3	0.086* ±0.031	42.1	**0.040** ±0.022**	**73.3**
E_7	0.13ns	34.6	0.082* ±0.028	44.8	0.063* ±0.019	58.4
E_8	0.08* ±0.030	23.7	0.086* ±0.025	42.6	**0.03** ±0.017**	**74.2**
E_9	0.07* ±0.026	28.2	0.072* ±0.021	52.3	**0.043** ±0.013**	**71.4**
E_{10}	0.090* ±0.043	32.9	0.071* ±0.019	51.3	0.047* ±0.018	68.2
E_{11}	0.089* ±0.031	30.3	0.065* ±0.02	56.8	**0.042** ±0.011**	**72.5**
E_{12}	0.11ns	26.3	0.061* ±0.028	55.6	0.06* ±0.029	60.2
Ascorbic acid	0.069* ±0.025	54.3	0.049* ±0.015	67.4	0.031** ±0.012	85.3

One way ANOVA followed by Dennett's't' test, *P<0.01, **P<0.001, ns- non significant

DISCUSSION AND CONCLUSION

Synthesized compounds were tested for antidiabetic, anti-inflammatory and antioxidant activity. In antidiabetic activity compounds **E_1, E_3, E_6, E_7, E_{11},** and **E_{12}** have shown more promising results. Few synthesized compounds have shown good anti-inflammatory action, amongst **E_{10}** has shown excellent activity at 600 µg/mL concentration. Observed good anti-inflammatory activity may be due to 4-OCH_3, 4-Cl and 4-NO_2 in the same compound. Compounds **E_1, E_6, E_8,** and **E_9** have shown promising antioxidant activity at 250 µg/mL. This observation may promote a further development of this group of pyrimidine and thiazolidinone may lead to compounds with better pharmacological profile than standard anti diabetic, anti-inflammatory and antioxidant drugs.

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