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RESEARCH ARTICLE

SYNTHESIS AND BIOLOGICAL EVALUATION OF CLUBBED TRIAZOLE-THIAZOLIDINONE DERIVATIVES

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In the present work, sixteen novel 3-(2-(4-(4-substituted benzylideneamino)-5-phenyl-4*H*-1,2,4-triazol-3-ylthio)acetyl)-2-(arylimino)thiazolidin-4-one (10a-p) derivatives were synthesized by clubbing 3-(2-chloroacetyl)-2-(arylimino)thiazolidin-4-one derivatives (4a-h) with SH group of triazole Schiff bases (9a-b). The structures of the newly synthesized compounds were confirmed by analytical and spectral methods (^{1}H NMR and IR). The biological potential of newly synthesized compounds was investigated through hydrogen peroxide scavenging assay, anthelmintic activity and *in vitro* α-amylase inhibition activity. Among synthesized compounds, 10l showed the most potent hydrogen peroxide scavenging activity 71.44%, 72.32% and 73.99% at different concentrations 10 μg/ml, 30 μg/ml, and 50 μg/ml respectively. Compound 10f showed the most potent anthelmintic activity with mean paralysis time 5.20±0.05 min and mean death time 7.64±0.16 min. Highest α-amylase inhibition activity 92.17% was exhibited by compound 10j.

Key words: Thiazolidin-4-one, 1,2,4-triazoles, Schiff's base, Hydrogen peroxide scavenging assay.

INTRODUCTION

Heterocyclic systems have been enriched with pharmacological activities since ancient times (Kumar, 2011; Mehta and Pathak, 2011; Dahiya and Mourya, 2013; Pareta *et al* 2013; Nusrat *et al* 2014). Among the heterocyclics, thiazolidin-4-one is a biologically important scaffold known to be associated with several biological activities such as antimicrobial (Deep *et al* 2014), anticancer (Wang *et al* 2012), antidiabetic (Ottana *et al* 2012) analgesic, anti-inflammatory (Deep *et al* 2012), hydrogen peroxide scavenging activity (Sharma *et al* 2011) and anticonvulsant activity (Kaur *et al* 2012) etc. Another five membered heterocycles like 1,2,4-triazole and their derivatives constitute an important class of compounds which act as a core nucleus of various therapeutically important drugs. Triazole derivatives also exhibit a broad array of agricultural, industrial and biological activities. The synthesis of these heterocycles has received considerable attention in recent years and several 1,2,4-triazole derivatives have been reported for diverse biological activities. Schiff bases of 1,2,4-triazoles have also been found to possess extensive biological activities (Kharb *et al* 2011; Singhal *et al* 2011; Chandermauli *et al* 2012).

Design of new bioactive agents with the development of hybrid molecules through the combination of different pharmacophores in the same structure may lead to compounds having more efficiency in biological response. The interesting biological properties of these two classes of compounds inspired us to synthesize new compounds with both moieties clubbed together with a hope of improved biological potential. Hence in the present study, we report

the synthesise and characterization of novel thiazolidin-4-one derivatives clubbed with 1,2,4-triazole moiety and biological evaluation of these compounds for hydrogen peroxide scavenging assay, anthelmintic and α-amylase inhibition activities (Wahi and Singh, 2011).

MATERIALS AND METHODS

General

Chemicals and all solvents used in the present work were procured from SD Fine Chem. Ltd., Sigma Aldrich Ltd. and Rankem Ltd. Melting points were determined on a Lab India MR-VIS visual melting range apparatus (Mumbai, India) and are uncorrected. Thin layer chromatography (TLC) was performed to monitor the reactions and to determine the purity of the products using TLC plates pre-coated with silica gel G employing methanol: chloroform (3:1) solvent system and UV light for visualization. The infrared (IR) spectra were recorded on a Shimadzu IR Spectrophotometer. ^{1}H NMR spectra were recorded using Bruker Avance II (Fallanden, Switzerland) 400MHz spectrometer with DMSO-d_6 as solvent and tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed as δ (ppm).

Chemistry

Synthesis of aryl thioureas (2a-h)

Aryl thioureas **2a-h** were prepared by adding concentrated hydrochloric acid (25 ml) to substituted anilines **1a-h** (25 ml) and the solution was warmed. A saturated solution of ammonium thiocyanate in water (30 g in 60 ml) was added slowly in above solution. The mixture was boiled until the solution got turbid. The turbid solution was poured in cold water. Aryl thioureas were separated as precipitates and then filtered. Further purification was carried out by re-crystallization with aqueous ethanol (80%) (Bhusari *et al* 2008).

Synthesis of 2-arylimino-1,3-thiazolidin-4-one derivatives (3a-h)

Aryl thioureas **2a-h** (0.04 mole, 8 g) were dissolved in 16.45 mL ethanol (95%). The resulting mixture was refluxed with fused sodium acetate (0.052 mole, 4.31 g) and ethylchloroacetate (5.65 ml, 6.46 g) for 4 to 5 h. The reaction mixture was then poured into ice cold water. The reaction mixture was kept overnight for complete precipitation and then filtered. The precipitates thus obtained were dried at room temperature and further purification was carried out by re-crystallisation in ethanol (95%) (Singh *et al* 2011).

Synthesis of 3-(2-chloroacetyl)-2-(arylimino) thiazolidin-4-one derivatives (4a-h)

A solution of 2-arylimino-1,3-thiazolidin-4-one **3a-h** (0.01 mol, 1.92 g) in 40 ml dry benzene was cooled to 0-5°C. Chloroacetyl chloride (0.01 mol, 1.12 ml) was added with vigorous stirring, followed by addition of triethylamine (4 ml) and potassium carbonate (0.01 mol, 1.38 g). The reaction mixture was then refluxed for 4–5 h. At the completion of reaction, the excess of benzene was distilled off. The residue was washed with 5 ml of water. An oily product was obtained which was separated out from water. The product obtained was stored in a cool environment (Shiradkar *et al* 2007).

Synthesis of benzoic acid hydrazide (6)

Benzoic acid hydrazide was prepared by adding excess of hydrazine hydrate (4 ml) to methyl benzoate **5** (0.01 mole, 1.36 g) and the resulting mixture was refluxed for 10 h. After refluxing, the mixture was poured into crushed ice to obtain white precipitates. These precipitates were filtered, washed with water and dried at room temperature. Further purification was carried out by re-crystallisation in ethanol (Parmar *et al* 2011).

Synthesis of potassium dithiocarbazinate (7)

The freshly prepared benzoic acid hydrazide **6** (0.01 mole, 1.36 g) was added to a solution of KOH (1.6 g) in ethanol (50 ml) and the resulting mixture was cooled on ice bath. Carbon disulphide was added (0.013 mole, 2.3 g) and the mixture was stirred for 10 h. After 10 h of stirring, the mixture was diluted with diethyl ether (30 ml) and stirred for further 1 h. potassium dithiocarbazinate was obtained as white salt on the bottom of flask.

Synthesis of 4-amino-5-phenyl-4H-1,2,4-tri azole-3-thiol (8)

To potassium dithiocarbazinate salt **7** (0.01 mol) without any purification, hydrazine hydrate (5 ml) in excess was added and diluted with water (20 ml) with stirring. The resulting mixture was refluxed gently (3 to 5 h) until the reaction mixture changed to deep green. During reflux, hydrogen sulphide was evolved. The reaction mixture was then cooled to 5°C on ice bath and acidified with conc. HCl to pH 1. A yellow solid separated out which was filtered, washed with

water and dried. Purification was done by re-crystallisation from ethanol.

Synthesis of triazole Schiff's bases (9a-b)

Triazole Schiff's bases **9a-b** were prepared by adding corresponding aldehyde (0.01 mol) in ethanol (25 ml), resulting mixture was treated with concentrated HCl (0.5 ml) and refluxed for 4-6 h. After refluxing, the reaction mixture was poured into ice cold water, filtered and dried. Further purification was carried out by re-crystallisation in ethanol (El-Sayed, 2006).

Synthesis of 3-(2-(4-(4-substituted benzylid-eneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio) acetyl)-2-(arylimino)thiazolidin-4-one (10a-p)

Equimolar solution of triazole Schiff's bases **9a-b** (0.01 mol) and 3-(2-chloroacetyl)-2-(arylimino) thiazolidin-4-one derivatives **4a-h** (0.01 mol) in dry benzene (30 ml) in the presence of anhydrous potassium carbonate and triethyl-amine (4.5 ml) was stirred at room temperature for 6 h. The mixture was filtered and the filtrate was evaporated to dryness. The residue was washed with water and re-crystallized from ethanol for further purification (Singh *et al* 2011). By adopting these procedures, sixteen novel compounds were synthesized.

Physical and analytical data of synthesized compounds is given in **Table 1**. Synthetic pathway for preparation of title compounds is shown in **Figure 1**.

Table 1. Structural and physiochemical data of thiazolidin-4-one derivatives 10(a-p)

Sr. No.	Ar	R	Mol. formula	Mol. weight	R_f value	Yield (%)
10a	C_6H_5	H	$C_{26}H_{20}N_6O_2S_2$	512.6	O.75	56.75
10b	C_6H_5	4-OCH_3	$C_{27}H_{22}N_6O_3S_2$	542.6	0.58	46.21
10c	2-NO_2-C_6H_4	H	$C_{26}H_{19}N_7O_4S_2$	557.6	0.45	48.56
10d	2-NO_2-C_6H_4	4-OCH_3	$C_{27}H_{21}N_7O_5S_2$	587.6	0.67	42.95
10e	4-NO_2-C_6H_4	H	$C_{26}H_{19}N_7O_4S_2$	557.6	0.64	16.18
10f	4-NO_2-C_6H_4	4-OCH_3	$C_{27}H_{21}N_7O_5S_2$	587.6	0.56	18.90
10g	2,4-$(NO_2)_2$-C_6H_3	H	$C_{26}H_{18}N_8O_6S_2$	602.6	0.69	36.60
10h	2,4-$(NO_2)_2$-C_6H_3	4-OCH_3	$C_{27}H_{20}N_8O_7S_2$	632.6	0.66	36.45
10i	4-OCH_3-C_6H_4	H	$C_{27}H_{22}N_6O_3S_2$	542.6	0.62	33.20
10j	4-OCH_3-C_6H_4	4-OCH_3	$C_{28}H_{24}N_6O_4S_2$	572.6	0.49	28.02
10k	2,4-$(CH_3)_2$-C_6H_3	H	$C_{28}H_{24}N_6O_2S_2$	540.6	0.26	56.60
10l	2,4-$(CH_3)_2$-C_6H_3	4-OCH_3	$C_{29}H_{26}N_6O_3S_2$	570.6	0.74	43.93
10m	4-F-C_6H_4	H	$C_{26}H_{19}FN_6O_2S_2$	530.6	0.65	24.57
10n	4-F-C_6H_4	4-OCH_3	$C_{27}H_{21}FN_6O_3S_2$	560.5	0.69	46.51
10o	4-Br-C_6H_4	H	$C_{26}H_{19}BrN_6O_2S_2$	591.5	0.66	23.72
10p	4-Br-C_6H_4	4-OCH_3	$C_{27}H_{21}BrN_6O_3S_2$	621.5	0.35	64.51

3-(2-(4-(Benzylideneamino)-5-phenyl-4H-1,2, 4-triazol-3-ylthio)acetyl)-2-(phenylimino) thiazolidin-4-one (10a)

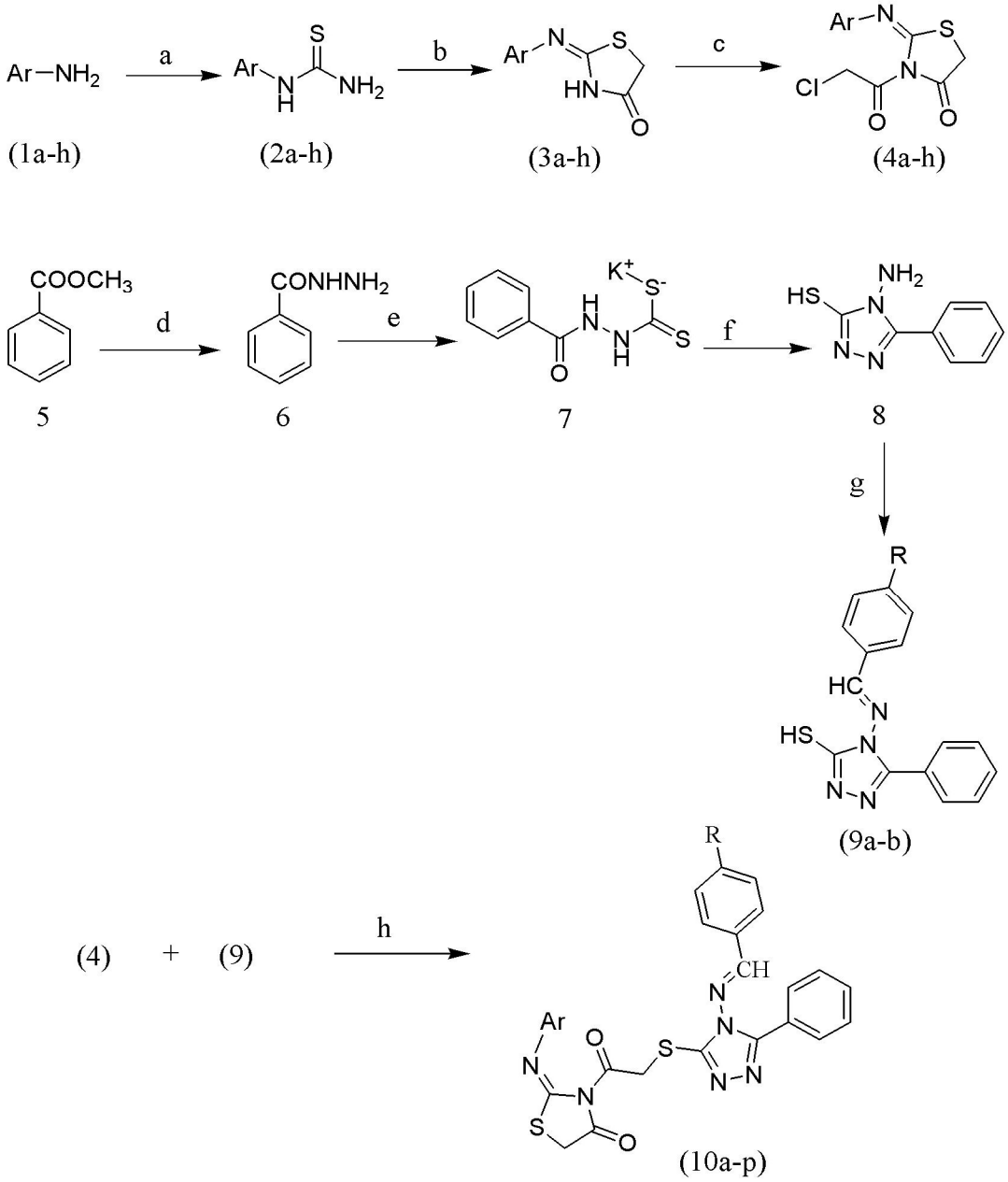
(Semisolid mass, yield: 56.75 %); ^{1}H NMR (DMSO-d_6) δ ppm**:** 8.06-8.15 (s, 1H, =CH), 7.08-7.71 (m, 15H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 4.01-4.07 (s, 2H, -CH_2-S), 3.76-3.91 (s, 2H, -CH_2); IR cm^{-1}: 1705 (C=O *str*), 1683 (C=O amide *str*), 1590 (N=CH *str*), 1485 (N-N str).

3-(2-(4-(4-Methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(phenylimino)thiazolidin-4-one (10b)

(Semisolid mass, yield: 42.21 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.91-7.96 (s, 1H,=CH), 7.02-7.87 (m, 14H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 4.07-4.08 (s, 3H, -OCH_3), 3.80-3.91 (s, 2H, -CH_2), 3.97-4.01 (s, 2H, -CH_2-S); IR cm^{-1}: 1710 (C=O *str*), 1680 (C=O amide *str*), 1589 (N=CH *str*), 1479 (N-N *str*), 1112 (C-O *str*).

3-(2-(4-(Benzylideneamino)-5-phenyl-4H-1,2, 4-triazol-3-ylthio)acetyl)-2-(2-nitrophenyl-imino)thiazolidin-4-one (10c)

(Semisolid mass, yield: 48.56 %); ^{1}H NMR (DMSO-d_6) δ ppm: 8.18-8.23 (s, 1H, =CH), 7.22-8.11 (m, 14H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 4.18-4.25 (s, 2H, -CH_2-S), 3.58-3.79 (s, 2H, -CH_2); IR cm^{-1}: 1702 (C=O *str*), 1681 (C=O amide *str*), 1587 (N=CH *str*), 1542 (NO_2 *str*), 1486 (N-N *str*).



Reagent and Reaction Conditions: (a) NH_4SCN, HCl Heating (b) Fused CH_3COONa, $ClCH_2COOC_2H_5$, Reflux 4-5 h.(c) Dry benzene, $ClCH_2COCl$, TEA.,K_2CO_3, Reflux 4-5 h. (d)$NH_2NH_2H_2O$, Reflux 10 h (e) CS_2 , KOH ,EtOH, Diethylether, Stir 10h (f) $NH_2NH_2H_2O$ Reflux4-5h.(g) Ethanol, Conc. HCl, ArCHO, Reflux 4-5 h. (h) Dry benzene, Anhydrous. K_2CO_3, Triethylamine, Stir for 6h.

Figure 1. Preparation of title compounds **10a-p**

3-(2-(4-(4-Methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(2-nitrophenylimino)thiazolidin-4-one (10d)

(Semisolid mass, yield: 42.95 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.92-8.16 (s, 1H, =CH), 7.12-7.81 (m, 13H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 4.07-4.08 (s, 3H, -OCH_3), 3.87-4.21 (s, 2H, -CH_2-S), 3.83-3.97 (s, 2H, -CH_2); IR cm^{-1}: 1711 (C=O *str*), 1682 (C=O amide *str*), 1591 (N=CH *str*), 1541 (NO_2 *str*), 1480 (N-N *str*), 1113 (C-O *str*).

3-(2-(4-(Benzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(4-nitrophenyl imino)thiazolidin-4-one (10e)

(Semisolid mass, yield: 16.18 %); ^{1}H NMR (DMSO-d_6) δ ppm: 8.28-8.45 (s, 1H, =CH), 7.21-7.91 (m, 14H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 4.11-4.20 (s, 2H, -CH_2-S), 3.42-3.69 (s, 2H, -CH_2); IR cm^{-1}: 1709 (C=O *str*), 1683 (C=O

amide *str*), 1586 (N=CH *str*),1539 (NO_2 *str*), 1481 (N-N *str*).

3-(2-(4-(4-Methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(4-nitrophenylimino)thiazolidin-4-one (10f)

(Semisolid mass, yield: 18.90 %); ^{1}H NMR (DMSO-d_6) δ ppm: 8.00-8.04 (s, 1H, =CH), 6.90-7.79 (m, 13H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 3.76-3.88 (s, 2H, -CH_2-S), 3.65-3.74 (s, 2H, -CH_2), 3.57 (s, 3H, -OCH_3); IR cm^{-1}: 1707 (C=O *str*), 1682 (C=O amide *str*), 1576 (N=CH *str*), 1548 (NO_2 *str*), 1480 (N-N *str*), 1108 (C-O *str*).

3-(2-(4-(Benzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(2,4-dinitrophenylimino)thiazolidin-4-one (10g)

(Semisolid mass, yield: 36.60 %); ^{1}H NMR (DMSO-d_6) δ ppm: 8.16-8.27 (s, 1H, =CH), 7.50-7.94 (m, 13H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 3.93-4.13 (s, 2H, -CH_2-S), 3.40-3.76 (s, 2H, -CH_2); IR cm^{-1}: 1704 (C=O *str*), 1681 (C=O amide *str*), 1587 (N=CH *str*), 1544 (NO_2 *str*), 1482 (N-N *str*).

3-(2-(4-(4-Methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(2,4-dinitrophenylimino)thiazolidin-4-one (10h)

(Semisolid mass, yield: 36.45 %); ^{1}H NMR (DMSO-d_6) δ ppm: 8.11-8.16 (s, 1H, =CH), 7.43-7.94 (m, 12H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 3.85-4.26 (s, 2H, -CH_2-S), 3.68-4.12 (s, 3H, -OCH_3).3.57-3.69 (s, 2H, -CH_2); IR cm^{-1}: 1711 (C=O str), 1680 (C=O amide *str*), 1590 (N=CH *str*), 1546 (NO_2 *str*), 1479 (N-N *str*), 1106 (C-O *str*).

3-(2-(4-(Benzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(4-methoxyphenylimino)thiazolidin-4-one (10i)

(Semisolid mass, yield: 33.20 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.83-7.85 (s, 1H, =CH), 6.87-7.33 (m, 14H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 4.10-4.20 (s, 2H, -CH_2-S), 3.75-4.10 (s, 3H, -OCH_3), 3.75-3.80 (s, 2H, -CH_2); IR cm^{-1}: 1710 (C=O *str*), 1683 (C=O amide *str*), 1591 (N=CH *str*), 1482 (N-N *str*), 1111 (C-O *str*).

3-(2-(4-(4-Methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(4-methoxyphenylimino)thiazolidin-4-one (10j)

(Semisolid mass, yield: 28.02 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.88-7.97 (s, 1H, =CH), 6.99-7.82 (m, 13H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 4.00-4.31 (s, 2H, -CH_2-S), 3.44-3.86 (s, 6H, -OCH_3), 3.44-3.77 (s, 2H, -CH_2); IR cm^{-1}: 1710 (C=O *str*), 1680 (C=O amide *str*), 1590 (N=CH *str*), 1482 (N-N *str*), 1108 (C-O *str*).

3-(2-(4-(Benzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(2,4-dimethylphenylimino)thiazolidin-4-one (10k)

(Semisolid mass, yield: 56.60 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.97-8.22 (s, 1H, =CH), 7.01-7.90 (m, 13H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 4.11-4.20 (s, 2H, -CH_2-S), 3.83-3.91 (s, 2H, -CH_2) 1.97-2.50 (s, 6H, -CH_3); IR (KBr) cm^{-1}: 1704 (C=O *str*), 1682 (C=O amide *str*), 1590 (N=CH *str*), 1486 (N-N *str*).

3-(2-(4-(4-Methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(2,4-dimethylphenylimino)thiazolidin-4-one (10l)

(Semisolid mass, yield: 43.93 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.97-8.22 (s, 1H, =CH), 7.34-7.80 (m, 12H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 3.84-4.09 (s, 2H, -CH_2-S), 3.01-4.09 (s, 3H, -OCH_3), 3.72-3.81 (s, 2H, -CH_2), 2.34.-2.82 (s, 6H, -CH_3); IR cm^{-1}: 1715 (C=O *str*), 1679 (C=O amide *str*), 1586 (N=CH *str*), 1475 (N-N *str*), 1106 (C-O *str*).

3-(2-(4-(Benzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(4-fluorophenylimino)thiazolidin-4-one (10m)

(Semisolid mass, yield: 24.75 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.93-8.25 (s, 1H, =CH), 7.14-7.83 (m, 14H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 3.69-4.27 (s, 2H, -CH_2-S), 3.56-3.79 (s, 2H, -CH_2); IR cm^{-1}: 1708 (C=O *str*), 1679 (C=O amide *str*), 1590 (N=CH *str*), 1483 (N-N str).

3-(2-(4-(4-Methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(4-fluorophenylimino)thiazolidin-4-one (10n)

(Semisolid mass, yield: 46.51 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.95-8.15 (s, 1H, =CH), 7.04-7.88 (m, 13H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 3.86-4.17 (s, 2H, -CH_2-S), 3.40-3.78 (s, 2H, -CH_2), 3.40-3.71 (s, 3H, -OCH_3); IR cm^{-1}: 1714 (C=O str), 1678 (C=O amide *str*), 1588 (N=CH *str*), 1478 (N-N *str*), 1114 (C-O *str*).

3-(2-(4-(Benzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(4-bromophenylimino)thiazolidin-4-one (10o)

(Semisolid mass, yield: 23.72 %); ^{1}H NMR

(DMSO-d_6) δ ppm: 7.96-8.13 (s, 1H, =CH), 6.73-7.90 (m, 14H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 3.84-4.00 (s, 2H, -CH_2-S), 3.67 -3.84 (s, 2H, -CH_2); IR cm^{-1}**:** 1706 (C=O *str*), 1683 (C=O amide *str*), 1590 (N=CH *str*), 1483 (N-N *str*).

3-(2-(4-(4-Methoxybenzylideneamino)-5-phenyl-4H-1,2,4-triazol-3-ylthio)acetyl)-2-(4-bromophenylimino)thiazolidin-4-one (10p)

(Semisolid mass, yield: 64.51 %); ^{1}H NMR (DMSO-d_6) δ ppm: 7.87-8.24 (s, 1H, =CH), 6.79-7.76 (m, 13H, -CH, Ar-Thiazolidinone, Triazole, Benzylidine), 3.91-4.21 (s, 2H, -CH_2-S), 3.74 -3.81 (s, 2H, -CH_2), 3.40-3.71 (s, 3H, -OCH_3); IR cm^{-1}: 1712 (C=O *str*), 1680 (C=O amide *str*), 1587 (N=CH *str*), 1486 (N-N *str*), 1115 (C-O *str*).

Biological Methods

All the newly synthesized compounds **10a-p** was tested for their *in vitro* hydrogen peroxide scavenging assay, anthelmintic activity and *in vitro* α-amylase inhibition activity. The effect of test compounds was compared with the standard drugs.

Evaluation method of scavenging of hydrogen peroxide (H_2O_2)

Antioxidant activity of all synthesized compounds **10a-p** has been evaluated by using hydrogen peroxide scavenging assay. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (10, 30 and 50 μg/ml) of all the synthesized compounds in DMSO were prepared. Hydrogen peroxide solution (0.6 ml, 40 mM) was added in the test and standard solutions of different concentrations.

Absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging of hydrogen peroxide of compound and standard compounds was calculated using the following formula

$$\% \text{ Scavenging } [H_2O_2] = [(A_0-A_1)/A_0] \times 100$$

where A_0 = absorbance of the control, and A_1 = absorbance in the presence of the sample and standards. Butylated hydroxyl anisole (BHA) and ascorbic acid were used as standards (Ciftci *et al* 2011).

Anthelmintic activity

Synthesized derivatives were tested for their ability to kill or expel roundworms or parasites from the body. For the experimentation purpose, six earthworms (*Pheretima posthuma*) of almost equal size (4-5 cm in length and 0.1-0.2 cm in width) were taken in standard drug solution and test compound solution. Both standard drug (Albendazole) and test compounds were dissolved in the minimum quantity of DMF (*N,N*-Dimethylformamide) and final concentration was made up to 0.2% *w/v* with the help of normal saline solution. Earthworms tested in normal saline (0.9% *w/v*) were taken as control group. Albendazole was used as standard drug. For the evaluation of the anthelmintic potential of the drug, time taken for complete paralysis of the earthworm and death was studied. The mean lethal time for each test compound was recorded and compared with standard drug. Time at which the earthworm became completely motionless was taken as paralysis time and death time was the time when they died. To ascertain the death of motionless worms, they were immersed in the water at 50°C and failure of earthworm to respond to this stimulus was considered as the death time (Vijay Kumar *et al* 2010; Suresh *et al* 2011).

α-Amylase inhibition activity

α-amylase is the enzyme responsible for the breakdown of oligo and disaccharides to monosaccharides. It catalyse breakdown of starch to maltose and finally to glucose. Inhibition of amylase enzymes involved in digestion of carbohydrates can significantly decrease the post prandial increase of blood glucose after a mixed carbohydrate diet. Inhibition of amylase enzyme can be an important strategy in management of blood glucose for the treatment of diabetes mellitus type II (Narkhede *et al* 2011). The starch solution (0.5% *w/v*) was prepared by stirring and boiling 0.25 g of soluble potato starch in 50 ml of deionised water for 15 min. The enzyme solution (0.5 unit/ml) was prepared by mixing 0.001 g of α-amylase in 100 ml of 20 mM sodium phosphate buffer (pH 6.9). The colour reagent was a solution containing 96 mM 3,5-dinitrosalicylic acid (DNSA) (20 ml), 5.31 M sodium potassium tartrate in 2 M sodium hydroxide (8 ml) and de-ionized water (12 ml). 23 mg of all synthesized compounds as test samples and acarbose in 1 ml of DMSO were dissolved. Acarbose was used as a standard at the concentration of 1 mg/ml. One ml of test solution in 1 ml enzyme solution were mixed in a

tube and incubated at 25°C for 30 min. 1 ml of starch solution was added to 1 ml of this mixture and incubated once again for 3 min at 25°C. Then, 1 ml of the colour 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, cooled and diluted with 9 ml distilled water and the absorbance value determined at 540 nm. Individual blanks were prepared for correcting the background absorbance. In this case, the colour reagent solution was added prior to the addition of starch solution and then the tube placed into the water bath. Controls were conducted in the same manner by replacing the drug sample with 1 ml DMSO. The inhibition percentage of *α*-amylase was calculated by the formula (Kumanan *et al* 2010; Nickavar and Yousefian, 2009):

$$\% \text{ Inhibiton} = \frac{\text{Absorbance (control) - Absorbance (test)}}{\text{Absorbance (control)}} \times 100$$

RESULT AND DISCUSSION

Chemistry

Novel 3-(2-(4-(4-substituted benzylidene-amino) -5-phenyl-4*H*-1,2,4-triazol-3-ylthio) acetyl)-2-(arylimino)thiazolidin-4-one **10a-p** derivatives were synthesized using three steps: synthesis of 3-(2-chloroacetyl)-2-(arylimino) thiazolidin-4-one derivatives **4a-h**, synthesis of triazole Schiff's bases 9(a-b) and finally clubbing of these derivatives. The compounds have been characterized by spectral methods such as IR and ^{1}HNMR. The proton NMR data of synthesized analogs **10a-p** shows multiplet at *δ* value ranging 6.7-8.5 ppm for the aromatic regions of both the rings, a singlet for (=CH) at *δ* value ranging 7.9–8.2 ppm, a characteristic peaks of a singlet of two protons of CH_2-S at *δ* value ranging 3.9 - 4.2 ppm for the clubbing, and a singlet of thiazolidin-4-one ring CH_2 group at *δ* value 3.7 ppm. The IR spectra of synthesized compounds **10a-p** displayed characteristic absorption peak in the region 1720-1705 cm^{-1} for C=O *str*, 1676-1667 cm^{-1} for CONH *str*, 1592-1575 cm^{-1} for N=CH *str* and 1550-1350 cm^{-1} for NO_2 *str*.

Hydrogen peroxide scavenging assay (H_2O_2)

Result from the hydrogen peroxide scavenging assay indicated that compounds having electron donating groups substitutions on aromatic region exhibited better hydrogen peroxide scavenging activity than compounds having electron withdrawing groups. Compounds **10l** (71.44%, 72.33% and 73.99%) and **10k** (72.14%, 74.31% and 76.72%) showed higher inhibition activity than standard ascorbic acid at different concentrations (10%, 30%, and 50%); their potency increased with the increase in concentration. The hydrogen peroxide scavenging of test compounds is tabulated in the **Table 2**.

Table 2. Hydrogen peroxide scavenging assay of the synthesized compounds **10a-p**

Compound	Scavenging of hydrogen peroxide at different concentration (%)		
	10 *μ*g/ml	30 *μ*g/ml	50 *μ*g/ml
10a	52.32	54.74	58.36
10b	55.61	56.25	58.78
10c	51.47	53.12	55.95
10d	52.83	53.51	54.29
10e	49.65	50.63	50.92
10f	50.45	52.31	53.17
10g	46.74	47.56	49.24
10h	47.81	49.24	50.37
10i	62.85	64.52	68.92
10j	61.3l	63.73	64.12
10k	71.44	72.32	73.91
10l	72.14	74.31	76.72
10m	32.45	33.23	35.93
10n	34.23	36.35	38.13
10o	42.51	45.22	46.84
10p	44.76	45.26	47.37
BHA	72.34	74.21	76.77
Ascorbic acid	63.51	64.89	67.32

Anthelmintic activity

All the synthesized compounds **10a-p** exhibited potent anthelmintic activity. Compounds having electron withdrawing group substitution showed better potency. Compound **10f** demonstrated most potent anthelmintic activity with mean paralysis time (5.20±0.05) min and mean death time (7.64±0.16) min followed by compound **10a** with mean paralysis time of (6.42±0.01) min and mean death time (8.35±0.05) min. Anthelmintic activity data is reported in **Table 3**.

Table 3. Anthelmintic evaluation of the synthesized compounds **10a-p**

Compound	Concentration (mg/ml)	Mean paralysis time (min)±S.E.	Mean death time (min)±S.E.
10a	0.2%	6.42±0.01	8.35±0.05
10b	0.2%	14.92±0.32	22.33±0.19
10c	0.2%	11.26±0.01	23.52±0.11
10d	0.2%	10.49±0.01	24.58±0.24
10e	0.2%	12.75±0.71	22.60±0.19
10f	0.2%	5.20±0.05	7.64±0.16
10g	0.2%	8.42±0.02	10.39±0.01
10h	0.2%	8.62±0.08**	12.48±0.20**
10i	0.2%	10.42±0.01	14.89±0.11*
10j	0.2%	14.12±0.008	21.93±0.11**
10k	0.2%	14.61±0.13	17.52±0.17
10l	0.2%	14.51±0.16**	16.37±0.15
10m	0.2%	14.39±0.05	17.21±0.10*
10n	0.2%	15.86±0.14*	25.15±0.03
10o	0.2%	15.19±0.16***	27.29±0.44
10p	0.2%	14.89±0.10*	26.61±0.09
Control	--	--	--
Albendazole	0.2%	41.45±0.28**	72.15±0.37

α-Amylase inhibition activity

All the tested compounds showed good inhibition of α-amylase percentage. Compounds with electron withdrawing group substitution showed the better inhibition. Compound **10j** was found most potent having 92.17% inhibiton of α-amylase followed by compound **10c** having 84.24% inhibition. The percentage inhibition shown by the synthesized compounds is tabulated in **Table 4**.

Table 4. *α*-Amylase inhibition activity of synthesized compounds

Compound	Concentration (mg/ml)	Inhibition (%)
10a	23	62.90
10c	23	84.24
10d	23	67.47
10h	23	64.77
10i	23	74.55
10j	23	92.17
10k	23	46.78
10l	23	53.77
10m	23	–
10o	23	71.94
Acarbose	1	89.76

CONCLUSION

In the present work, sixteen novel 3-(2-(4-(4-substituted benzylideneamino)-5-phenyl-4*H*-1,2,4-triazol-3-ylthio) acetyl)-2-(arylimino) thiazolidin-4-one derivatives **10a-p** were synthesized with good yield. Structures of newly synthesized compounds were characterized by ^{1}H NMR and IR spectral data. Synthesized

compounds were screened for *in vitro* antioxidant, anthelmintic and *α*-amylase inhibition activity. Results of biological evaluation were found to be encouraging. These compounds can be explored for possible utility as potent therapeutic agents. In future, combination of thiazolidin-4-one and triazoles can be explored for the search of newer drugs for various diseases and infections.

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