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ORIGINAL ARTICLE

Synthesis of 3-aryl-4-({2-[4-(6-substituted-coumarin-3-yl)-1,3-thiazol-2-yl]hydrazinylidene}methyl/ethyl)-sydnones using silica sulfuric acid and their antidiabetic, DNA cleavage activity

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Abstract A novel one-pot synthesis of sydnones appended to coumarins (**4a–r**) *via* thiazole in presence of silica sulfuric acid as a heterogeneous catalyst is discussed. The use of low cost and reusable silica sulfuric acid as catalyst makes this process feasible and convenient. Further, the title compounds were screened for their α-amylase inhibition (antidiabetic) as well as DNA cleavage activities.

1. Introduction

Sydnones have gained importance not only due to their structural features as 1,3-dipoles, but also because of their biological significance. During the past, many interesting data have been obtained on the structures (Cooper et al., 2005; Hasek et al., 1979), reactivities (Dumitrascu et al., 2002; Cherepanov and Kalinin, 2000), physicochemical pharmacological properties (Sasaki and Ishibashi, 1990; Handa et al., 1997; Moustafa et al., 2004; Dunkley and Thoman, 2003) of sydnones. Coumarin nucleus has been the aim of many workers as most of its derivatives were proved to be active as antitumor, antibacterial, antifungal, anticoagulant and antiinflammatory agents (Raev et al., 1990; El-Agrody et al., 2001; Emmanuel-Giota et al., 2001). On the other hand thiazole derivatives have been reported to be biologically versatile compounds displaying wide range of antiinflammatory, anticancer, antibacterial, antifungal and antiallergic properties (Kennedy and Thornes, 1997; Andrewi et al., 1996; Anu et al., 1977; Roscoe et al., 1971; Martin et al., 1997).

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α-Amylase is an important enzyme in the body responsible for hydrolyzing polysaccharides such as dietary starch. Inhibition of this enzyme could be extremely beneficial in diabetic patients because it could lower the glucose levels in the blood. One of the therapeutic approaches adopted thus far to ameliorate postprandial hyperglycemia involves the retardation of glucose absorption *via* the inhibition of carbohydrate-hydrolyzing enzymes *viz*., α-glycosidase and α-amylase in the digestive organs (Bhandari et al., 2008). The powerful synthetic α-glucosidase and α-amylase inhibitors such as acarbose, miglitol and voglibose, function directly in reducing the sharp increases in glucose levels that occur immediately after food intake (Saito et al., 1998; Sels et al., 1999; Stand et al., 1999). However, the continuous use of these synthetic agents should be limited because these agents may induce side effects such as flatulence, abdominal cramps, vomiting and diarrhoea (Hanefeld, 1998). Additionally, there have been some reports describing an increased incidence of renal tumors, serious hepatic injury and acute hepatitis (Diaz-Gutierrez et al., 1998; Charpentier et al., 2000). Therefore, a number of studies have been conducted in the search for naturally derived α-glycosidase and α-amylase inhibitors that induce no deleterious side effects (Matsui et al., 2007; Kim et al., 2008; Heo et al., 2009).

Several synthetic methods have been reported for the preparation of thiazoles (Hantzsch and Weber, 1887; Hantasch, 1888; Dodson and Kingh, 1945; King and Halvacek, 1950; Rajeswar Rao et al., 1996). Although some of these methods are useful, the majority of them suffer from at least one of the disadvantages such as low yield, prolonged reaction time, use of toxic organic solvents, excess of reagents and catalysts, high cost and susceptibility to moisture. In recent years, evolution of chemical reactions involving less hazardous, environmentally acceptable and recyclable catalytic systems have gained considerable attention both in industry and academia (Wang et al., 2006; Ando et al., 1982; Kabalka et al., 1999, 2001; Hosseinzadeh et al., 2008; Silva et al., 2008; Boruah et al., 2007; Ge and Hu, 2007; Movassagh and Shokri, 2005; yadav et al., 2001; Blass et al., 1999; Blass, 2002). Generally, the solid acid catalysts are mainly based on clay and silica (Cornelis and Laszlo, 1994; Clark and Marquarie, 1998; Varma et al., 1999). In terms of convenience, silica based catalysts are inexpensive, easy to prepare and insoluble in most of the organic solvents so as to recover and recycle from the reactions. Further, this reagent is safe, easy to handle, non-toxic, environmentally benign and presents fewer disposal problems. In view of this, silica sulfuric acid has been used as a solid acid catalyst in many reactions such as nitration of aromatic compounds (Riego et al., 1996), oxidation of thiols to disulfides (Zolfigol, 2001), three component Biginelli reaction (Salehi et al., 2003), preparation of diacetates (Hajipour et al., 2005) and deprotection of tetrahydropyranyl ethers (Hajipour et al., 2006) acetals and ketals (Mirjalili et al., 2002). However, to the best of our knowledge, the synthesis of sydnone derivatives containing thiazole and coumarin entities using silica sulfuric acid as a catalyst has not been reported so far.

In continuation of our work aiming at the synthesis of sydnone derivatives appended to coumarin, we herein report a green reaction of thiosemicarbazone (**2a**–**f**), with 3-bromoacetyl-(6-substituted)-coumarin (**3g**–**i**) at a time in dry ethanol using catalyst silica sulfuric acid to afford a series of thiazole derivatives (**4a**–**r**) appended to coumarin and sydnone. Because, it was envisaged that the two pharmacophores if linked together would generate novel molecular templates which are likely to exhibit interesting biological properties in animal models. The present study also includes testing of target compounds for their antidiabetic activity (α-amylase inhibition) and DNA cleavage analyses.

2. Experimental

2.1. General

Melting points were determined in open capillaries. IR (KBr) spectra were recorded on FT-IR spectrometer. ^{1}H NMR spectra ($CDCl_3$) were recorded using Varian-300 MHz FT-NMR spectrometer with TMS as an internal standard. Mass spectra were recorded on GCMS-SC/AD/17-004 instrument. Silica sulfuric acid was prepared according to the reported method (Zolfigol, 2001).

2.2. General methods of preparation of 4-({2-[4-(6-substituted-coumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}-methyl/ethyl)-3-arylsydnones (*4a-r*)

2.2.1. Method 1

3-Aryl-4-formyl/acetylsydnone (**1a-f**) (0.10 mol) dissolved in alcohol was added into a solution of thiosemicarbazide (0.20 mol) in hot water. The contents were stirred for 5 min and the precipitate thus formed was separated and filtered. Recrystalization from 50% aqueous alcohol yielded the crystals of (**2a-f**). The compound (**2a-f**) was then refluxed with equimolar quantity of (6-substituted)-3-bromoacetylcoumarin (**3g-i**) and silica sulfuric acid (5 mol%) in dry ethanol for an appropriate time (as indicated in Table 1). After completion of the reaction the catalyst was removed by filtration and the filtrate was poured into ice cold water. The crude product (**4a-r**) formed was filtered and recrystallized from ethanol to obtain yellow crystals.

Table 1 Synthesis of thiazole derivatives.

Compounds	Method 1		Method 2		M.P. (°C)
	Time (h)	Yield (%)	Time (h)	Yield (%)	
4a	4.10	56	3.00	80	184–85 [a]
4b	5.10	60	2.50	72	233–35 [a]
4c	4.30	52	2.00	74	228–29
4d	4.40	55	2.35	68	212–13 [a]
4e	5.15	58	2.45	75	223–24 [a]
4f	4.50	50	2.15	71	216–17
4g	5.00	54	2.30	73	196–97
4h	5.35	62	3.15	81	258–59
4i	4.40	58	2.55	76	240–41
4j	5.25	56	3.20	69	173–74
4k	5.05	49	3.00	75	206–07
4l	4.00	53	2.20	79	191–92
4m	4.20	57	2.50	73	156–57
4n	5.10	55	3.30	78	213–14
4o	5.30	52	2.35	72	179–80
4p	4.20	50	3.10	76	188–89
4q	5.15	53	3.40	80	244–45
4r	4.20	57	2.35	81	217–18

[a] Kalluraya and Rai (2004).

2.2.2. Method 2

A mixture of 3-aryl-4-formyl/acetylsydnone (**1a-f**) (0.01 mol), thiosemicarbazide (0.01 mol) and 3-bromoacetyl-(6-substituted)-coumarin (**3g-i**) (0.01 mol), was dissolved in dry ethanol (50 ml) and was refluxed with silica sulfuric acid (5 mol%). The progress of the reaction was monitored by TLC and after completion of the reaction (Table 1), the catalyst was filtered and the solvent was removed under vacuo to yield the crude product, which was then recrystallized from ethanol to get the needles of (**4a-r**).

*2.2.2.1. 4-({2-[4-(Coumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}methyl)-3-phenylsydnone (**4a**).* Yield 80%, m.p. 184–185 °C. IR (KBr): υ 3445 (NH), 1738 (sydnone C═O), 1720 (coumarin C═O), 1612 (C═N) cm^{-1}. ^1H NMR (300 MHz, CDCl3, 25 °C, TMS): δ = 7.35 (s, 1H, $C_{5'}$–H), 7.38–7.95 (m, 10H, Ar–H), 8.48 (s, 1H, H–C═N) ppm; MS: m/z = 433. Anal. calcd for $C_{21}H_{13}N_5O_4S$: C, 58.46; H, 3.04; N, 16.23. Found: C, 58.10; H, 3.00; N, 16.18.

*2.2.2.2. 4-({2-[4-(6-Bromocoumarin-3-yl)-1,3-thiazol-2-yl]hydrazinylidene}methyl)-3-phenyl sydnone (**4b**).* Yield 72%, m.p. 233–234 °C. IR (KBr): υ 3448 (NH), 1740 (sydnone C═O), 1725 (coumarin C═O), 1618 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.10 (s, 1H, $C_{5'}$–H), 7.30–7.82 (m, 10H, Ar–H), 8.41 (s, 1H, H–C═N) ppm; MS: m/z = 515 (M^{+2}, 18), 513 (M^+, 22). Anal. calcd for $C_{21}H_{12}BrN_5O_4S$: C, 49.42; H, 2.37; N, 13.72. Found: C, 49.35; H, 2.40; N, 13.68.

*2.2.2.3. 4-({2-[4-(6-Chlorocoumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}methyl)-3-phenylsydnone (**4c**).* Yield 74%, m.p. 196–197 °C. IR (KBr): υ 3446 (NH), 1738 (sydnone C═O), 1721 (coumarin C═O), 1615 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 7.00 (s, 1H, $C_{5'}$–H), 7.40–7.88 (m, 10H, Ar–H), 8.35 (s, 1H, H–C═N) ppm; MS: m/z = 471 (M^{+2}, 7), 469 (M^+, 20). Anal. calcd for $C_{21}H_{12}ClN_5O_4S$: C, 54.14; H, 2.60; N, 15.03. Found: C, 54.10; H, 2.56; N, 14.96.

*2.2.2.4. 4-({2-[4-(Coumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}methyl)-3-p-tolylsydnone (**4d**).* Yield 68%, m.p. 212–213 °C. IR (KBr): υ 3452 (NH), 1746 (sydnone C═O), 1728 (coumarin C═O), 1610 (C═N) cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.8 (s, 3H, CH_3), 6.55 (s, 1H, $C_{5'}$–H), 7.00–7.70 (m, 10H, Ar–H), 8.20 (s, 1H, H–C═N) ppm; MS: m/z = 446. Anal. calcd for $C_{22}H_{15}N_5O_4S$: C, 59.32; H, 3.39; N, 15.72. Found: C, 59.22; H, 3.34; N, 15.68.

*2.2.2.5. 4-({2-[4-(6-Bromocoumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}methyl)-3-p- tolylsydnone (**4e**).* Yield 75%, m.p. 223–224 °C. IR (KBr): υ 3458 (NH), 1749 (sydnone C═O), 1718 (coumarin C═O), 1622 (C═N) cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 3.00 (s, 3H, CH_3), 6.15 (s, 1H, $C_{5'}$–H), 7.10–7.80 (m, 8H, Ar–H), 8.28 (s, 1H, H–C═N) ppm; MS: m/z = 528 (M^{+2}, 13), 526 (M^+, 17). Anal. calcd for $C_{22}H_{14}BrN_5O_4S$: C, 50.39; H, 2.69; N, 13.36. Found: C, 50.30; H, 2.62; N, 13.30.

*2.2.2.6. 4-({2-[4-(6-Chlorocoumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}methyl)-3-p-tolylsydnone (**4f**).* Yield 71%, m.p. 236–237 °C. IR (KBr): υ 3453 (NH), 1745 (sydnone C═O), 1727 (coumarin C═O), 1618 (C═N) cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.90 (s, 3H, CH_3), 6.35 (s, 1H, $C_{5'}$–H), 7.15-7.75 (m, 8H, Ar–H), 8.25 (s, 1H, H–C═N) ppm; MS: m/z = 484 (M^{+2}, 12), 482 (M^+, 34). Anal. calcd for $C_{22}H_{14}ClN_5O_4S$: C, 54.83; H, 3.35; N, 14.53. Found: C, 54.78; H, 3.30; N, 14.49.

*2.2.2.7. 3-(p-Chlorophenyl)-4-({2-[4-(coumarin-3-yl)-1,3-thiazol-2-yl]hydrazinylidene}methyl)-sydnone (**4g**).* Yield 73%, m.p. 208–209 °C. IR (KBr): υ 3447 (NH), 1742 (sydnone C═O), 1722 (coumarin C═O), 1615(C═N) cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.45 (s, 1H, $C_{5'}$–H), 7.40–7.94 (m, 9H, Ar–H), 8.44 (s, 1H, H–C═N) ppm; MS: m/z = 469 (M^{+2}, 9), 467 (M^+, 25). Anal. calcd for $C_{21}H_{14}ClN_5O_4S$: C, 53.91; H, 3.02; N, 14.97. Found: C, 53.85; H, 2.95; N, 14.90.

*2.2.2.8. 4-({2-[4-(6-Bromocoumarin-3-yl)-1,3-thiazol-2-yl]hydrazinylidene}methyl)-3-p-chlorophenylsydnone (**4h**).* Yield 81%, m.p. 216–217 °C. IR (KBr): υ 3449 (NH), 1744 (sydnone C═O), 1723 (coumarin C═O), 1623 (C═N) cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.20 (s, 1H, $C_{5'}$–H), 7.42–8.00 (m, 8H, Ar–H), 8.38 (s, 1H, H–C═N) ppm; MS: m/z = 549 (M^{+4}, 11), 547 (M^{+2}, 35), 545 (M^+, 27). Anal. calcd for $C_{21}H_{11}ClBrN_5O_4S$: C, 46.13; H, 2.40; N, 12.81. Found: C, 46.08; H, 2.35; N, 12.80.

*2.2.2.9. 4-({2-[4-(6-Chlorocoumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}methyl)-3-p-chlorophenylsydnone (**4i**).* Yield 76%, m.p. 211–212 °C. IR (KBr): υ 3447 (NH), 1743 (sydnone C═O), 1726 (coumarin C═O), 1620 (C═N) cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 6.30 (s, 1H, $C_{5'}$–H), 7.38–7.94 (m, 8H, Ar–H), 8.31 (s, 1H, H–C═N) ppm; MS: m/z = 505 (M^{+4}, 9), 503 (M^{+2}, 22), 501 (M^+, 36); Anal. calcd for $C_{21}H_{11}Cl_2N_5O_4S$: C, 50.41; H, 2.22; N, 14.00. Found: C, 50.37; H, 2.20; N 13.96.

*2.2.2.10. 4-(1-{2-[4-(Coumarin-3-yl)-1,3-thiazol-2-yl]hydrazinylidene}ethyl)-3-phenylsydnone (**4j**).* Yield 69%, m.p. 191–192 °C. IR (KBr): υ 3428 (NH), 1732 (sydnone C═O), 1728 (coumarin C═O), 1595 (C═N), cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.42 (s, 3H, CH_3), 7.41 (s, 1H, $C_{5'}$–H), 7.66–8.60 (m, 10H, Ar–H), 11.61 (s, 1H, NH) ppm; MS: m/z = 445. Anal. calcd for $C_{22}H_{15}N_5O_4S$: C, 59.32; H, 3.39; N, 15.72. Found: C, 59.28; H, 3.34; N, 15.70.

*2.2.2.11. 4-(1-{2-[4-(6-Bromocoumarin-3-yl)-1,3-thiazol-2-yl]hydrazinylidene}-ethyl)-3-phenylsydnone (**4k**).* Yield 75%, m.p. 226–227 °C. IR (KBr): υ 3435 (NH), 1738 (sydnone C═O), 1724 (coumarin C═O), 1610 (C═N) cm^{-1}, ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.39 (s, 3H, CH_3), 7.24 (s, 1H, $C_{5'}$–H), 7.52–8.18 (m, 9H, Ar–H), 10.88 (s, 1H, NH) ppm; MS: m/z = 527 (M^{+2}, 22.40), 525 (M^+, 27.30). Anal. calcd for $C_{22}H_{14}BrN_5O_4S$: C, 50.39; H, 2.69; N, 13.36. Found: C, 50.35; H, 2.65; N, 13.34.

*2.2.2.12. 4-(1-{2-[4-(6-Chlorocoumarin-3-yl)-1,3-thiazol-2-yl]hydrazinylidene}ethyl)-3-phenylsydnone (**4l**).* Yield 79%, m.p. 203–204 °C. IR (KBr): υ 3432 (NH), 1735 (sydnone C═O), 1730 (coumarin C═O), 1600 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.27 (s, 3H,

CH_3), 7.28 (s, 1H, $C_{5'}$–CH), 7.36–8.12 (m, 9H, Ar–H), 11.12 (s, 1H, NH) ppm; MS: m/z = 483 (M^{+2}, 12.60), 481 (M^+, 34.80); Anal. calcd for $C_{22}H_{14}ClN_5O_4S$: C, 55.06; H, 2.94; N, 14.59. Found: C, 55.00; H, 2.90; N, 14.55.

*2.2.2.13. 4-(1-{2-[4-(Coumarin-3-yl)-1,3-thiazol-2-yl]hydrazinylidene}ethyl)-3-p-tolylsydnone (**4m**).* Yield 73%, m.p. 219–220 °C. IR (KBr): υ 3440 (NH), 1740 (sydnone C═O), 1726 (coumarin C═O), 1621 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.35 (s, 3H, CH_3), 2.39 (s, 3H, Ar–CH_3), 6.8 (s, 1H, $C_{5'}$–H), 7.05–7.77 (m, 9H, Ar–H), 8.25 (s, 1H, NH) ppm; MS: m/z = 458. Anal. calcd for $C_{23}H_{17}N_5O_4S$: C, 60.12; H, 3.73; N, 15.24. Found: C, 60.08; H, 3.70; N, 15.20.

*2.2.2.14. 4-(1-{2-[4-(6-Bromocoumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}ethyl)-3-p-tolylsydnone (**4n**).* Yield 78%, m.p. 179–180 °C. IR (KBr): υ 3448 (NH), 1742 (sydnone C═O), 1729 (coumarin C═O), 1636 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.37 (s, 3H, CH_3), 2.32 (s, 3H, Ar–CH_3), 6.50 (s, 1H, $C_{5'}$–H), 7.15-7.96 (m, 8H, Ar–H), 8.22 (s, 1H, NH) ppm; MS: m/z = 540 (M^{+2}, 23.40), 538 (M^+, 28.10). Anal. calcd for $C_{23}H_{16}BrN_5O_4S$: C, 51.31; H, 3.00; N, 13.01. Found: C, 51.25; H, 2.96; N, 12.98.

*2.2.2.15. 4-(1-{2-[4-(6-Chlorocoumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}ethyl)-3-p-tolylsydnone (**4o**).* Yield 72%, m.p. 243–244 °C. IR (KBr): υ 3446 (NH), 1740 (sydnone C═O), 1725 (coumarin C═O), 1632 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.36 (s, 3H, CH_3), 2.42 (s, 3H, Ar–CH_3), 6.65 (s, 1H, $C_{5'}$–H), 7.10–7.82 (m, 8H, Ar–H), 8.26 (s, 1H, NH) ppm; MS: m/z = 496 (M^{+2}, 10.20), 494 (M^+, 29.30). Anal. calcd for $C_{23}H_{16}ClN_5O_4S$: C, 55.93; H, 3.27; N, 14.18. Found: C, 55.90; H, 3.22; N, 14.15.

*2.2.2.16. 4-(1-{2-[4-(Coumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}ethyl)-3-p-chlorophenylsydnone (**4p**).* Yield 76%, m.p. 188–189 °C. IR (KBr): υ 3431 (NH), 1743 (sydnone C═O), 1724 (coumarin C═O), 1631 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.70 (s, 3H, CH_3), 6.70 (s, 1H, $C_{5'}$–H), 7.20–7.73 (m, 9H, Ar–H), 8.44 (s, 1H, NH) ppm; MS: m/z = 483 (M^{+2}, 8.60), 481 (23.50). Anal. calcd for $C_{22}H_{14}ClN_5O_5S$: C, 55.06; H, 2.94; N, 14.59. Found: C, 55.00; H, 2.90; N, 14.55.

*2.2.2.17. 4-(1-{2-[4-(6-Bromocoumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}ethyl)-3-p-chlorophenylsydnone (**4q**).* Yield 80% m.p. 215–216 °C, IR (KBr): υ 3439 (NH), 1748 (sydnone C═O), 1729 (coumarin C═O), 1642 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.45 (s, 3H, CH_3), 6.40 (s, 1H, $C_{5'}$–H), 7.32–7.92 (m, 8H, Ar–H), 8.45 (s, 1H, NH) ppm; MS: m/z = 565 (M^{+4}, 9), 563 (M^{+2}, 29), 561 (M^+, 24). Anal. calcd for $C_{22}H_{13}BrClN_5O_4S$: C, 47.29; H, 2.34; N, 12.53. Found: C, 47.25; H, 2.30; N, 12.50.

*2.2.2.18. 4-(1-{2-[4-(6-Chlorocoumarin-3-yl)-1,3-thiazol-2-yl]-hydrazinylidene}ethyl)-3-p-chlorophenylsydnone (**4r**).* Yield 81%, m.p. 204–205 °C. IR (KBr): υ 3435 (NH), 1744 (sydnone C═O), 1722 (coumarin C═O), 1640 (C═N) cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.50 (s, 3H, CH_3), 6.50 (s, 1H, $C_{5'}$–H), 7.30–7.88 (m, 8H, Ar–H), 8.40 (s, 1H, NH) ppm; MS: m/z = 520 (M^{+4}, 10), 518 (M^{+2}, 26), 516 (M^+, 40). Anal. calcd for $C_{22}H_{13}Cl_2N_5O_4S$: C, 51.37; H, 2.55; N, 13.62. Found: C, 51.33; H, 2.50; N, 13.57.

2.3. Amylase inhibition assay (Sadasivam and Manickam, 1996)

The activity of amylase (Himedia, Mumbai) was assayed with different concentrations of sample along with different volumes of solvent, with control. The test tubes were added with sodium phosphate buffer (1 ml, 50 mM, pH 7.0–7.3), different volumes of solvent, different concentrations of sample, starch (0.5 ml, in buffer) and enzyme (0.5 ml, from 1 mg/ml sample in buffer). The blank tube was added with DNS (1 ml) before adding enzyme. The tubes were incubated at 37 °C for 10 min followed by addition of DNS (0.1 ml). The tubes were incubated in boiling water bath for 10 min, cooled and read for absorbance at 540 nm against blank. The maltose liberated was determined by the help of standard maltose curve and activities were calculated according to the formula,

$$\text{Activity} = \frac{\text{Conc.of Maltose liberated} \times \text{Volume of enzyme used (ml)}}{\text{Mol.wt of maltose} \times \text{incubation times (min)}} \times \text{Dilution factor}$$

The inhibitory/induction property shown by the sample was compared with that of control and expressed as percent induction/inhibition. This was calculated according to the relation,

$$\%\ \text{Inhibition/Induction} = \frac{\text{Activity in presence of compound}}{\text{Control activity}}$$

2.4. DNA cleavage activity

2.4.1. Preparation of culture media

DNA cleavage experiments were done according to the literature (Sambrook et al., 1989). Nutrient broth [peptone, 10; yeast extract, 5; NaCl, 10; in (g/l)] was used for culturing the pathogen *Escherichia coli*. Media (50 ml) was prepared, and autoclaved for 15 min at 121 °C under 15 lb pressures. The autoclaved media was inoculated for 24 h at 37 °C.

2.4.2. Isolation of DNA

The fresh bacterial culture (1.5 ml) was centrifuged to obtain the pellet which was then dissolved in lysis buffer (0.5 ml, 100 mM tris pH 8.0, 50 mM EDTA, 10% SDS), and saturated phenol (0.5 ml) was added and incubated at 55 °C for 10 min, then centrifuged at 10,000 rpm for 10 min and to the supernatant, equal volume of chloroform: isoamyl alcohol (24:1) and 1/20th volume of 3 M sodium acetate (pH 4.8) was added. Centrifuging at 10,000 rpm for 10 min and to the supernatant 3 volumes of chilled absolute alcohol was added. The precipitated DNA was separated by centrifugation and the pellet was dried and dissolved in TAE buffer (10 mM tris pH 8.0, 1 mM EDTA) and stored in cold condition.

2.4.3. Agarose gel electrophoresis

Cleavage products were analyzed by agarose gel electrophoresis method. Test samples (1 mg/ml) were prepared in DMF. The samples (25 mg) were added to the isolated DNA of *E. coli*. The samples were incubated for 2 h at 37 °C and then DNA sample (20 ml, mixed with bromophenol blue dye at 1:1 ratio) was loaded carefully into the electrophoresis chamber wells along with standard DNA marker containing TAE buffer (4.84 g tris base, pH 8.0, 0.5 M EDTA/1 l) and finally loaded on agarose gel and constant 50 V of electricity for 45 min was passed. The gel was then removed carefully and stained with ETBR solution (10 μg/ml) for 10–15 min and the bands were observed under UV transilluminator and then photographed to determine the extent of DNA cleavage. The results were compared with standard DNA marker.

3. Results and discussion

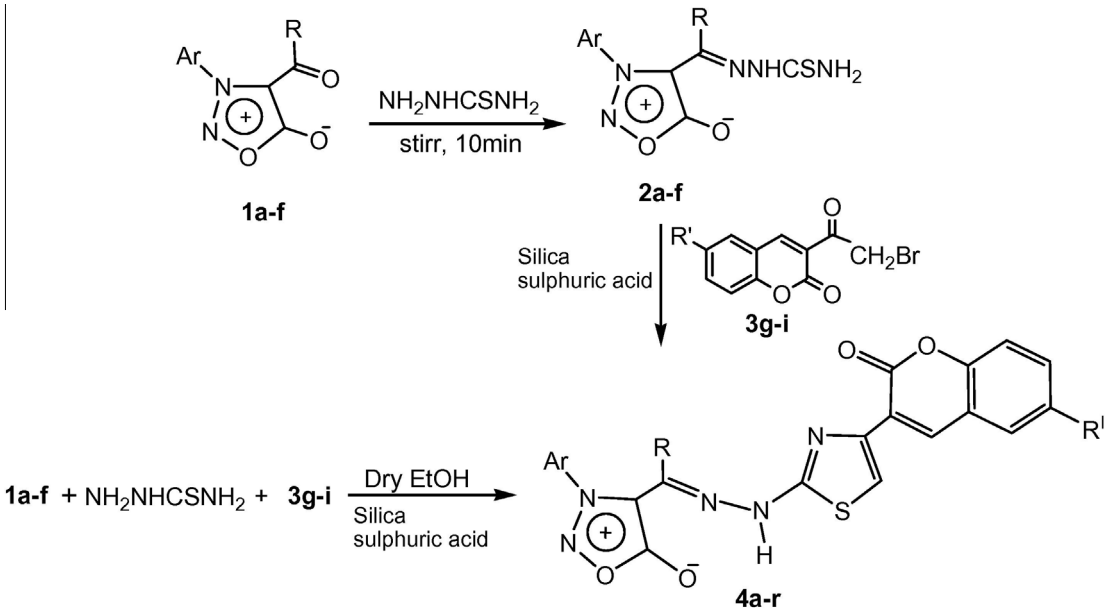
In method 1, thiosemicarbazones (**2a-f**) were prepared by the reaction of 3-aryl-4-formyl/acetylsydnone (**1a-f**) with thiosemicarbazide. The title compounds (**4a-r**) were then obtained by refluxing (**2a-f**) with 3-bromoacetyl-(6-substituted)-coumarin (**3g-i**) in the presence of silica sulfuric acid (5 mol%) in alcohol under reflux (about 10 h) with 50–60% yield.

The method 2 has a general applicability *viz*., to reduce all these steps and avoid wastage of chemicals for the preparation of title compounds (**4a-r**). A mixture of 3-aryl-4-formyl/acetylsydnone (**1a-f**), thiosemicarbazide and 3-bromoacetyl-6-substituted-coumarin (**3g-i**), was refluxed in the presence of silica sulfuric acid (5 mol%) in dry ethanol. The yield of the reaction was found to be higher than that of the method 1 and also time taken for the completion of the reaction was reduced to a greater extent (Table 1). It is one step three component synthesis (Scheme 1). The probable mechanism of formation of thiazole ring in the title compounds (**4a-r**) under the influence of catalyst silica sulfuric acid is given in the Scheme 2.

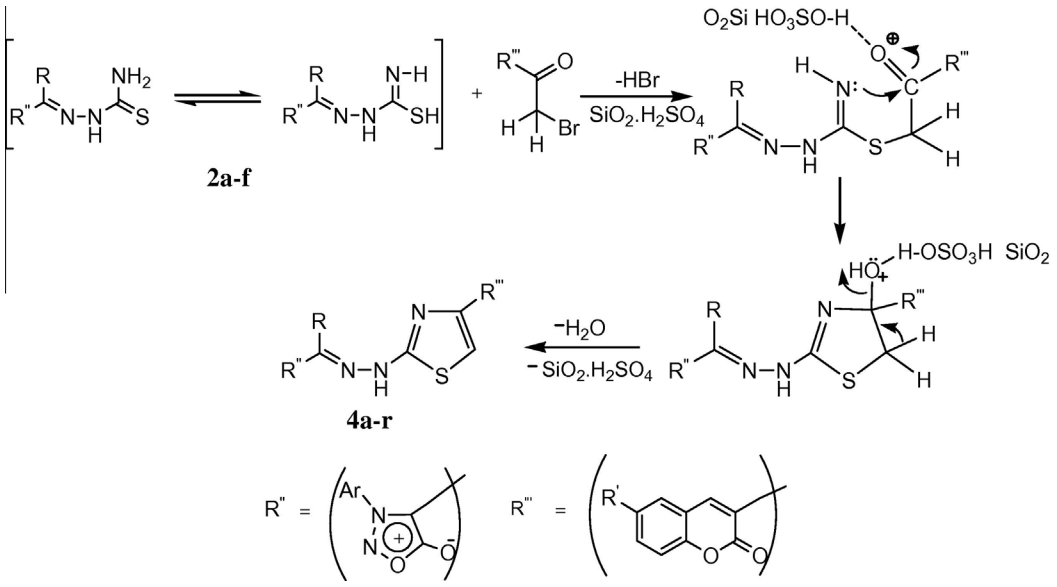
In comparison with the method 1, yield of the reaction under one-pot catalyzed condition was higher with a less time. Therefore, we employed the above conditions for preparation of the title compounds in the presence of silica sulfuric acid. The work-up with the use of catalyst is simple and the reusability of silica sulfuric acid was also analyzed by the separation and reloading in a new run and found that the catalyst could be reused several times without compromising in the productivity.

3.1. Antidiabetic activity

In this study, we have evaluated the inhibitory effects of title compounds (**4a-r**) against α-amylase to elucidate the possible use of title compounds (**4a-r**) as anti-hyperglycemic agents. The compounds (**4c**, **4h**, **4i** and **4r**) (with electron withdrawing groups) exhibited stronger inhibitory activity against α-amylase as compared to the control enzyme inhibitor. Whereas, the compounds (**4a**, **4g**, **4l** and **4p**) have shown moderate activities and rest of the compounds have shown weak inhibitory activity. The bar graph representation of comparative activities of title compounds (**4a-r**) is shown in Fig. 1.



Scheme 1 Formation of thiazole derivatives **4a-r**. **1a-f**/**2a-f**: **1a/2a:** Ar = C_6H_5, R = H; **1b/2b**: Ar = *p*-$CH_3C_6H_4$, R = H; **1c/2c**: Ar = *p*-ClC_6H_4, R = H; **1d/2d**: Ar = C_6H_5, R = CH_3; **1e/2e**: Ar = *p*-$CH_3C_6H_4$, R = CH_3; **1f/2f**: Ar = *p*-ClC_6H_4, R = CH_3, **3g-i**: **3g**: R′ = H; **3h**: R′ = Br; **3i**: R′ = Cl; **4a-r**: **4a**: Ar = C_6H_5, R = H, R′ = H; **4b**: Ar = C_6H_5, R = H, R′ = Br; **4c**: Ar = C_6H_5, R = H, R′ = Cl; **4d**: Ar = *p*-$CH_3C_6H_4$, R = H, R′ = H; **4e**: Ar = *p*-$CH_3C_6H_4$, R = H, R′ = Br; **4f**: Ar = *p*-$CH_3C_6H_4$, R = H, R′ = Cl; **4g**: Ar = *p*-ClC_6H_4, R = H, R′ = H; **4h**: Ar = *p*-ClC_6H_4, R = H, R′ = Br; **4i**: Ar = *p*-ClC_6H_4, R = H, R′ = Cl; **4j**: Ar = C_6H_5, R = CH_3, R′ = H; **4k**: Ar = C_6H_5, R = CH_3, R′ = Br; **4l**: Ar = C_6H_5, R = CH_3, R′ = Cl; **4m**: Ar = *p*-$CH_3C_6H_4$, R = CH_3, R′ = H; **4n**: Ar = *p*-$CH_3C_6H_4$,, R = CH_3, R′= Br; **4o**: Ar = *p*-$CH_3C_6H_4$, R = CH_3, R′ = Cl; **4p**: Ar = *p*-ClC_6H_4, R = CH_3, R′ = H; **4q**: Ar = *p*-ClC_6H_4, R = CH_3, R′ = Br; **4r**: Ar = *p*-ClC_6H_4, R = CH_3, R′ = Cl.



Scheme 2 Proposed mechanism for the formation of title compounds (**4a-r**) under the influence of silica sulfuric acid.

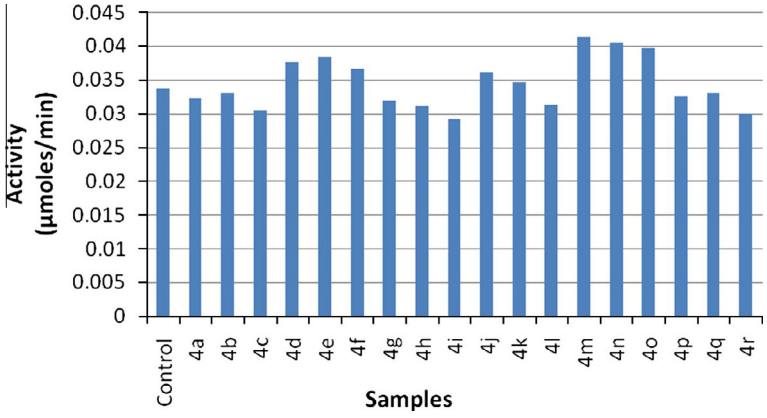


Figure 1 Amylase Inhibitory activity of Title compounds (**4a-r**)

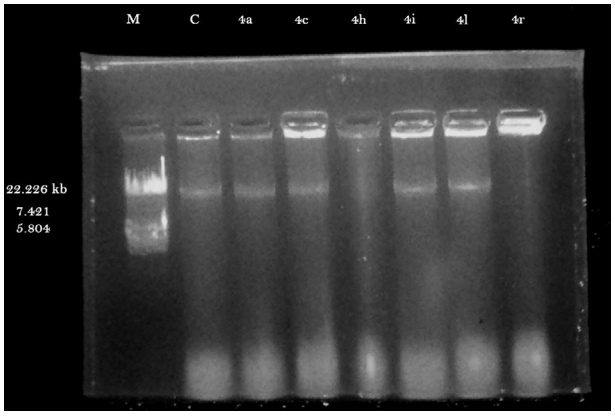


Figure 2 DNA cleavage activity of the title compounds

3.2. DNA cleavage activity

The DNA cleavage activity was determined using gel electrophoresis according to reported method (Sambrook et al., 1989). The picture of the gels is presented in Fig. 2. The gel after electrophoresis clearly revealed that, all tested compounds did act on the DNA as little tailing in the bands can be observed in treated samples. The difference was observed in the bands of all compounds as compared to the control DNA. This showed that the control DNA alone does not show any apparent cleavage as compounds did. With this, it can be concluded that the compounds inhibit the growth of the pathogenic organism by cleaving the genome.

4. Conclusion

In conclusion, we have demonstrated a new and efficient method for the synthesis of thiazole derivatised with coumarin and

sydnones using a catalytic amount of silica sulfuric acid. In contrast to the other catalysts, storage and handling of this catalyst did not require any special precautions. It could be stored for months with the same reactivity. The method involved in the present work addresses the current drive towards green chemistry due to low cost and easy availability of the catalyst, simple workup, high yield, easy handling and non-toxicity of the catalyst. The preliminary *in vitro* anti-diabetic activity of these novel series of thiazole derivatives has evidenced that some of the chlorine substituted (electron withdrawing group) compounds have exhibited good α-amylase inhibition and good DNA cleavage activity.

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