Arabian Journal of Chemistry (2016) 9, S225–S229

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Synthesis and antimicrobial activities of novel 1,4-benzothiazine derivatives

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Received 11 January 2011; accepted 5 March 2011
Available online 12 March 2011

KEYWORDS

2-Aminothiophenol;
1,4-Benzothiazine;
Ultrasound

Abstract A series of 2*H*,4*H*-2-[3,5-dimethyl-4-(substituted) phenyl azo pyrazol-1-yl] carbonyl methyl-3-oxo-1,4-benzothiazine derivatives have been synthesized by the reaction of 2*H*,4*H*-2-hydrazino carbonyl methyl-3-oxo-1,4-benzothiazine with acetyl acetone derivatives using ultrasound in lesser time with higher yields. All the synthesized compounds were investigated for their antibacterial activities. The result indicated that the compounds show convincing activities against Gram-positive bacteria (*Bacillus subtilis* and *Streptococcus lactis*) when compared with standard drug (ampicillin trihydrate). These compounds were also synthesized by conventional method and their structures have been elucidated on the basis of spectral analyses and chemical reactions.
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1. Introduction

1,4-Benzothiazine is the pharmacophore of phenothiazines, which are well established anti-psychotic drugs (Barker et al., 1969), and is also known as the basic unit for their utility as dyestuffs (Tanaka et al., 1966), photographic developers (Strain et al., 1946), ultraviolet light absorbers and antioxidants (Rasmussen, 1974).

The chemistry of pyrazole derivatives has been the subject of much research because of their importance in various applications and their widespread potential, biological and pharmacological activities, such as antimicrobial (Akbas et al., 2006), antiviral (Adnan et al., 2003), antitumor (Badwey et al., 1998), pesticidal (Londershausen et al., 1996) and analgesic (Bakavoli et al., 2006). It is also known that 1,4-benzothiazine is the pharmacophore of phenothiazines, which are well established anti-psychotic drugs (Barker et al., 1969), and is also known as the basic unit for their utility as dyestuffs (Tanaka et al., 1966), photographic developers (Strain et al., 1946), ultraviolet light absorbers and antioxidants (Rasmussen, 1974).

Now-a-days, application of ultrasound (sonochemistry) has become an exciting field of research. The chemical effects of ultrasound are diverse and include substantial improvements in both stoichiometric and catalytic chemical reactions. Ultrasonic irradiation accelerates the reactivity million fold and many synthetically useful reactions were successfully accomplished (Einhom et al., 1989). As compared to conventional conditions, viz. strong base and long reaction time, the ultrasonic irradiation procedure is milder and more conven-

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Peer review under responsibility of King Saud University.

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http://dx.doi.org/10.1016/j.arabjc.2011.03.009
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tional leading to higher yields in shorter reaction time (Mason, 1991).

Owing to the wide application of benzothiazines and their derivatives, rapid, safe, ecofriendly as well as economical method for the synthesis of 2*H*,4*H*-2-[3,5-dimethyl-4-(substituted) phenyl azo pyrazol-1-yl] carbonyl methyl-3-oxo-1,4-benzothiazine, is now reported.

2. Experimental

Melting points of all synthesized compounds were determined in open capillary tubes on an electro thermal apparatus and are uncorrected. The progress of reaction was monitored by TLC on silica gel coated aluminium plates (Merck) as adsorbents and UV light as a visualizing agent. IR spectra (potassium bromide in cm^{-1}) were recorded on a Perkin-Elmer spectrophotometer in the range of 4000–400 cm^{-1}. ^1H NMR spectra were recorded on Varian 500 MHz NMR spectrophotometer using deuterated dimethylsulfoxide as solvent and trimethylsilane as an internal standard (chemical shifts in δ ppm) and ms spectra were taken on a Jeol sx-102/PA-6000 (EI) spectrometer. C, H, N estimations were recorded on Carlo Erba 1108 (CHN) Elemental Analyser. Experiments under ultrasound irradiation was carried out in probe sonicator manufactured by Dakshin. (Model No. 230 V. AC, 50 Hz)

*2.1. 3,4-Dihydro-2-methoxycarbonylmethyl-3-oxo-2H-1,4-benzothiazine (**3**)*

2.1.1. Method A (ultrasound method)

2-Aminothiophenol (**1**) (1.25 g, 0.01 mol), maleic anhydride (**2**) (0.98 g, 0.01 mol), methanol (25 mL) and Conc. H_2SO_4 (98%, 2 mL) were taken in a 100 mL round bottomed flask and subjected to sonication for 10 min. After completion of the reaction (monitored by TLC) cooled solid thus obtained was washed with 5% sodium bicarbonate solution and extracted in dichloromethane to afford **3**.

2.1.2. Method B (conventional)

An equimolar mixture of compound **1** (0.01 mol), **2** (0.01 mol) and methanol (50 mL) was refluxed in the presence of Conc. H_2SO_4 (98%, 2 mL) for about 6–7 h. The product was isolated in a similar manner as in method A. The physical data are given in Table 1.

IR (cm^{-1}): (NH) 3274, (CH) 3010, (C═O) 1710, (C═O) 1640.

^1H NMR (DMSO-d_6, 500 MHz,): δ 2.887 (d, 2H, CH_2), 3.613 (s, 6H, CH_3), 3.854 (t, 1H, CH), 6.893–7.327 (m, 4H, ArH), 10.707 (s, 1H, ring NH).

*2.2. 2-Hydrazinocarbonylmethyl-3, 4-dihydro-3-oxo-2H-1, 4-benzothiazine (**4**)*

2.2.1. Method A (ultrasound method)

Compound (**3**) (2.37 g, 0.01 mol), hydrazine hydrate (2 g, 0.02 mol) and dry methanol (20 mL) were taken in a 100 mL round bottomed flask and subjected to sonication for 8 min. After completion of the reaction (monitored by TLC) cooled, poured on crushed ice, solid thus obtained was washed with water and recrystallised from methanol to get **4**.

2.2.2. Method B (conventional)

In a round bottomed flask (100 mL) fitted with a reflux condenser, a mixture of **3** (0.01 mol) and hydrazine hydrate (0.02 mol) in dry methanol (70 mL) was heated on a steam bath for 6 h. The reaction mixture was then concentrated, cooled and recrystallized to get the product. The physical data are given in Table 1.

IR (cm^{-1}): ($NH–NH_2$) 3310–3278, (CH) 3012, (C═O) 3310.

^1H NMR (DMSO-d_6, 500 MHz,): δ 2.694 (d, 2H, CH_2), 3.821 (t, 1H, CH), 4.224 (s, 2H, NH_2), 6.984–7.303 (m, 4H, ArH), 9.092 (s, 1H, NH), 10.657 (s, 1H, ring NH).

*2.3. 3-(4-Methylphenylazo)-2,5-pentadione **(5a)***

To an ice cold solution of *p*-toludine (0.02 mol) in a mixture of concentrated HCl (36%, 8 mL) and water (11 mL), a cold aqueous solution of sodium nitrite (1.40 g) was added in portions under ice cold condition. The diazonium salt so formed was then filtered into an already cooled (0 °C) solution containing sodium acetate (16 g) and acetyl acetone (0.02 mol) in ethanol (50 mL), then the solution was stirred vigorously. The separated solid was washed with water and recrystallised from ethanol. (Yield 75%, m.p. 109–111 °C). Similarly **5b–5g** were prepared. (**5b** m.p. 102–104 °C; 5c m.p. 113–115 °C; 5d m.p. 128–131 °C; 5e m.p. 117–119 °C).

*2.4. 2H,4H-2-[3,5-dimethyl-4-(methyl)-phenyl azo pyrazol-1-yl] carbonyl methyl-3-oxo-1,4-benzothiazine (**6a**)*

2.4.1. Method A (ultrasound method)

Compounds **4** (1.18 g, 0.005 mol), **5a** (1.32 g, 0.005 mol) and glacial acetic acid (20 mL) were taken in a 100 mL round

Table 1 Physical data of the synthesized compounds.

Compound	R	Molecular formula	Melting point (°C)	Yield%		Time	
				Son	Conv	Son (min)	Conv (h)
3	–	$C_{11}H_{11}NO_3S$	138–140	87	72	10	6
4	–	$C_{10}H_{11}N_3O_2S$	190–192	80	70	8	6
6a	CH_3	$C_{22}H_{21}N_5O_2S$	238–240	87	65	25	9
6b	OCH_3	$C_{22}H_{21}N_5O_3S$	218–220	84	62	27	8
6c	Cl	$C_{21}H_{18}N_5O_2SCl$	210–212	80	59	30	9
6d	NO_2	$C_{21}H_{18}N_6O_4S$	288–289	85	60	25	8
6e	H	$C_{21}H_{19}N_5O_2S$	269–270	82	64	25	8

Table 2 Antimicrobial activities of compounds **6a–6e**.

Compound	Inhibition zone (mm)						
	Gram-negative		Gram-positive		Fungi		Yeast
	E. coli	*P. putide*	*B. subtilis*	*S. lactis*	*A. niger*	*P.* sp.	*C. albicans*
6a	10	8	16	16	14	12	5
6b	8	8	14	14	10	10	5
6c	12	10	17	15	16	14	5
6d	7	5	12	11	10	12	5
6e	5	2	10	8	8	10	3
Ampicilin®	24	20	19	22	24	14	14

E. coli = *Escherichia coli*; *P. putide* = *Pseudomonas putide*; *B. subtilis* = *Bacillus subtilis*; *S. lactis* = *Sterptococcus lactis*; *A. niger* = *Aspergillus niger*; *P.* sp. = *Penicillium* sp.; *C. albicans* = *Candida albicans*.
The sensitivity of microorganisms to the tested compounds is identified in the following manner*.
Highly sensitive = Inhibition zone: 15–20 mm.
Moderately sensitive = Inhibition zone: 10–15 mm.
Slightly sensitive = Inhibition zone: 5–10 mm.
Not sensitive = Inhibition zone: 0 mm.
* Each result represents the average of triplicate readings.

bottomed flask and subjected to sonication for 24 min. Upon completion of the reaction (monitored by TLC) the reaction mixture was then cooled, poured into crushed ice. The precipitate was filtered and the solid was then crystallized from ethanol.

2.4.2. Method B (conventional)

An equimolar mixture of **4** and **5a** (0.005 mol) together with glacial acetic acid (50 mL) was refluxed in a round bottomed flask for 9 h. After conclusion of the reaction (TLC), the reaction mixture poured onto crushed ice; the solid mass that separated out was filtered, washed with water and crystallized with ethanol to give the desired compound **6a**. The physical data are given in Table 1.

IR (cm^{-1}): (NH) 3314, (CH) 3015, (C═O) 1660, (C═N) 1530.

^{1}H NMR (DMSO-d_6, 500 MHz,): δ 2.284 (s, 6H, $2\times CH_3$), 3.285 (d, 2H, CH_2), 3.872 (s, 1H CH), 6.785–7.717 (m, 9H, ArH), 10.862 (s, 1H ring NH).

^{13}C NMR (DMSO-d_6, 500 MHz,): δ 12.121 (CH_3), 20.932 (CH_3), 26.345 (CH_3), 33.052 (CH_2), 80.207 (CH), 121.232–122.451 (C═C), 123.175–139.541 (Ar–C), 158.524 (C═N), 161.637 (C═O), 167.108 (C═O).

MS (*m*/*z*): 420 (M^+), 235, 165.

2.5. 2H,4H-2-[3,5-dimethyl-4-(methoxy)-phenyl azo pyrazol-1-yl] carbonyl methyl-3-oxo-1,4-benzothiazine ***(6b)***

IR (cm^{-1}): (NH) 3310, (CH) 2984, (C═O) 1655, (C═N) 1580.

^{1}H NMR (DMSO-d_6, 500 MHz,): δ 2.181 (s, 6H, $2\times CH_3$), 3.172 (d, 2H, CH_2), 3.827 (s 3H, OCH_3), 4.122 (t 1H CH), 6.981-7.755 (m, 8H, ArH), 10.722 (s 1H ring NH).

^{13}C NMR (DMSO-d_6, 500 MHz,): δ 21.411 ($2\times CH_3$), 31.124 (CH_2), 55.474 (OCH_3), 84.253 (CH), 125.424–126.357 (C═C), 127.214–137.365 (Ar–C), 158.913 (C═N), 166.750 (C═O), 169.404 (C═O).

MS (*m*/*z*): 436(M^+), 329, 301, 235, 165.

2.6. 2H,4H-2-[3,5-dimethyl-4-(chloro)-phenyl azo pyrazol-1-yl] carbonyl methyl-3-oxo-1,4-benzothiazine ***(6c)***

IR (cm^{-1}): (NH) 3290, (CH) 2984, (C═O) 1660, (C═N) 1550, (C–Cl) 735.

^{1}H NMR (DMSO-d_6, 500 MHz,): δ 2.214 (s, 6H, $2\times CH_3$), 3.281 (d, 2H, CH_2), 4.117 (t, 1H, CH), 6.578–7.325 (m, 8H, ArH), 10.525 (s, 1H, ring NH).

^{13}C NMR (DMSO-d_6, 500 MHz,): δ 21.544 ($2\times CH_3$), 32.222 (CH_2), 79.561 (CH), 127.261–128.687 (C═C), 129.56–138.17 (Ar–C), 152.471 (C═N), 163.213 (C═O), 170.146 (C═O).

2.7. 2H,4H-2-[3,5-dimethyl-4-(nitro)-phenyl azo pyrazol-1-yl] carbonyl methyl-3-oxo-1,4-benzothiazine ***(6d)***

IR (cm^{-1}): (NH) 3287, (CH) 2994, (C═O) 1657, (C═N) 1544, (NO_2) 1360.

^{1}H NMR (DMSO-d_6, 500 MHz,): δ 2.253 (s, 6H, $2\times CH_3$), 2.914 (d, 2H, CH_2), 3.942 (t, 1H, CH), 7.125–7.688 (m, 8H, ArH), 10.621 (s, 1H, ring NH).

^{13}C NMR (DMSO-d_6, 500 MHz,): δ 21.114 ($2\times CH_3$), 30.513 (CH_2), 80.732 (CH), 128.101–129.784 (C═C), 130.254–142.325 (Ar–C), 150.335 (C═N), 167.544 (C═O), 169.104 (C═O).

2.8. 2H,4H-2-[3,5-dimethyl-4-phenyl azo pyrazol-1-yl] carbonyl methyl-3-oxo-1,4-benzothiazine ***(6e)***

IR (cm^{-1}): 3314 (NH), 3015 (CH), 1660 (C═O), 1530 (C═N).

^{1}H NMR (DMSO-d_6, 500 MHz,): δ 2.284 (s, 6H, $2\times CH_3$), 3.286 (d, 2H, CH_2), 3.871 (s, 1H, CH), 6.783–7.711 (m, 9H, ArH), 10.865 (s, 1H, ring NH).

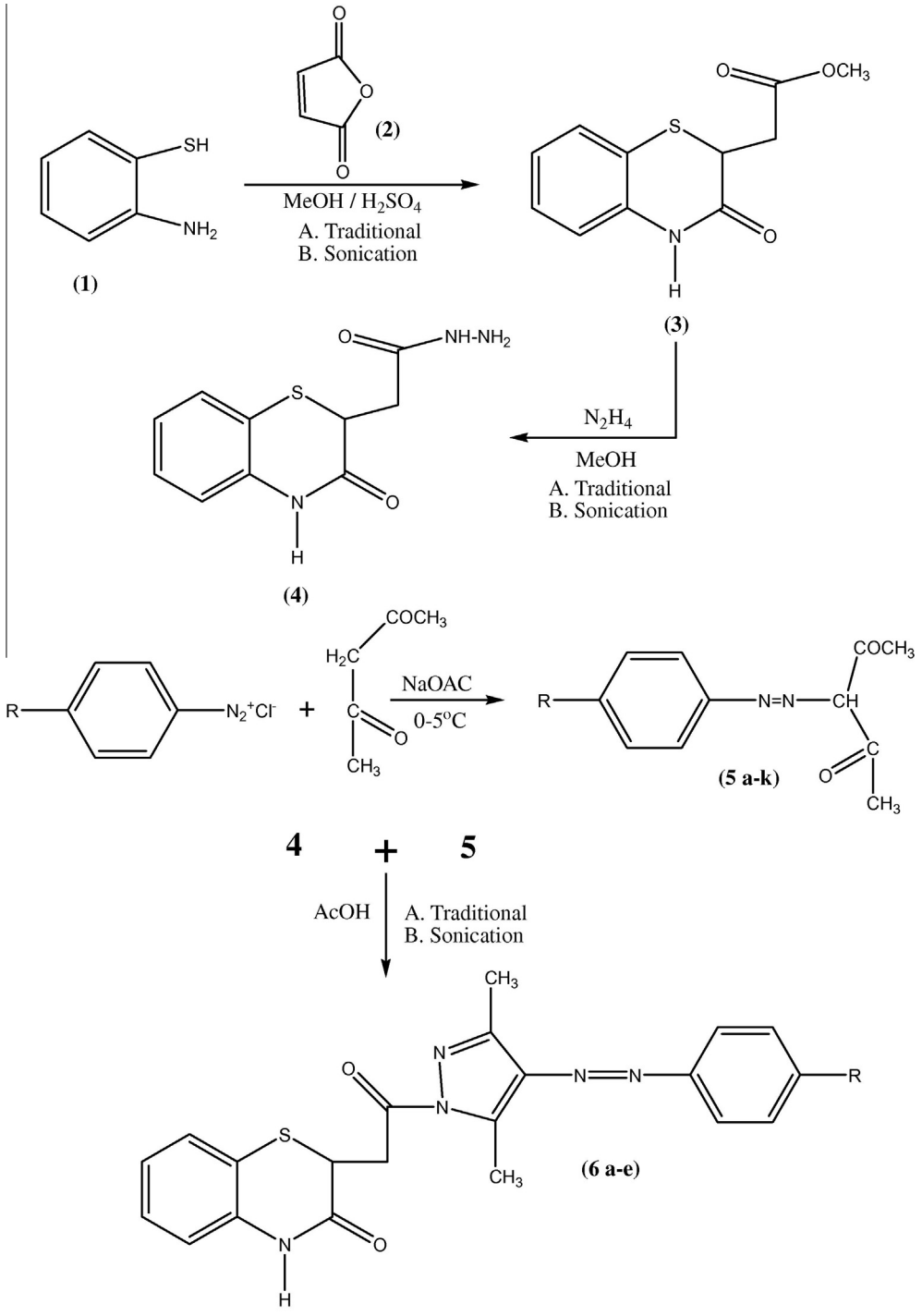
MS (*m*/*z*): 406 (M^+), 329, 207.

The physical data of the compounds are given in Table 1.

3. Biological evaluation

3.1. In vitro antimicrobial evaluation

The newly synthesized compounds, shown in Table 1 were tested for their antimicrobial activity against the following microorganisms: (a) Gram-negative: *Escherichia coli, Pseudomonas putide;* (b) Gram-positive: *Bacillus subtilis, Streptococcus lactis;* (c) Fungi: *Aspergillus niger, Penicillium* sp.; (d) Yeast:



Scheme 1 Synthesis of 1,4-benzothiazines.

Candida albicans. The preliminary screening of the investigated compounds was performed using the filter paper disc-diffusion method (Morrison et al.). The compounds were tested at a concentration of 100 μg/mL. The zone of inhibition was measured in mm and compared with reference standard ampicillin trihydrate (50 μg/mL). The compounds tested displayed good activity towards Gram positive bacteria, but were less active against Gram-negative bacteria. The results of antibacterial screening studies are reported in Table 2.

4. Result and discussion

2-Aminothiophenol (**1**) with an equimolar amount of maleic anhydride (**2**) in the presence of ethanol and conc. H_2SO_4 undergoes cyclization to form 3,4-dihydro-2-methoxycarbonylmethyl-3-oxo-2*H*-1,4-benzothiazine (**3**). The formation of (**3**) was explained on the basis of an IR band at 1740 and 1690 cm^{-1} due to ester and cyclic >C═O, respectively. This was also confirmed from ^{1}HNMR signal at 3.6 ppm due

to $-CH_3$. The compound (**3**) was subsequently reacted with hydrazine hydrate in dry methanol to form (**4**). The formation of compound **4** which was ascertained on the basis of IR bands at 3350, 3310 and 1630 due to NH–NH_2 and in ^{1}HNMR spectra disappearance of signal at 3.6 ppm due to $-CH_3$ and appearance of signal at 4.2 ppm and at 9.08 ppm due to NH_2 and –NH, respectively, confirms the formation of compound (**4**). The diazonium salt of primary amine when coupled with an acetyl acetone at 0–5 °C in the presence of sodium acetate afforded (**5**). Furthermore compound (**4**) undergoes cyclization with compound (**5**) to furnish 2*H*,4*H*-2-[3,5-dimethyl-4-(substituted) phenyl azo pyrazol-1-yl] carbonyl methyl-3-oxo-1,4-benzothiazine (**6**). (Scheme 1) The disappearance of signal at 4.2 ppm and the appearance of signals at 2.2 ppm and 2.8 due to $-CH_3$ are main indications that cyclization took place. Also the molecular ion peak in mass spectra supports the structure.

5. Conclusion

In conclusion, the ultrasound irradiation for synthesis of the title compounds offers reduction in reaction time, operation simplicity, cleaner reaction, easy work up and improved yields. The procedure clearly highlights the advantages of ultrasound. The synthesized compounds 1,4-benzothiazines showed promising antibacterial activity against Gram-positive bacteria. The data reported in this article may be a helpful guide for the medical chemists who are working in this area.

Acknowledgement

The authors are grateful to the Principal Ms. Manju J. Nichani and Management of K.C. College, Mumbai for providing necessary facilities. Authors are also thankful to the Director, Institute of Science, Mumbai for providing spectral analyses.

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