Acta Poloniae Pharmaceutica – Drug Research, Vol. 65 No. 5 pp. 521–526, 2008 ISSN 0001-6837
Polish Pharmaceutical Society

DRUG SYNTHESIS

SYNTHESIS AND BIOLOGICAL STUDIES OF BIS (THIADIAZOLE/TRIAZOLE) BY SONICATION

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Abstract: 5,5-Dimethylcyclohexane-1,3-dione was treated with semicarbazide to yield its acid hydrazide, which on further reaction under ultrasound condition with aryl isothiocyanates gave1,3-bis-imino-[1-(carboxy)-4-substituted phenylthiosemicarbazide]-5,5-dimethylcyclohexane. This compound in acidic medium gave 1,3-bis-imino-[5-(substituted) phenylamino-1,3,4-thiadiazol-2-yl-]-5,5-dimethylcyclohexane, whereas in basic medium 1,3-bis-imino-[4-(substituted) phenyl-5-mercapto-1,2,4-triazol-3-yl-]-5,5-dimethylcyclohexane was obtained. The synthesized compounds were investigated for their antibacterial activities. The results indicated that the compounds show convincing activities against Gram-positive bacteria (*S. aureus*, *C. diphtheriae* and *S. cerevisiae*) 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.

Keywords: semicarbazide, isothiocynates, thiadiazoles, triazoles

Research on a new substance possessing antibacterial activity has attracted considerable attention owing to the continuous increase in the bacterial resistance (1). Further, infection caused by various microorganisms pose a serious challenge to the medical community and need for an effective therapy has led to the search for novel antibacterial agents (2). The pharmacologically important heterocycles with nitrogen bridge derived from 1,2,4-triazole paved the way toward active research in triazole chemistry. As a result, a variety of new improved compounds were being added to this field every year. A number of attempts were made to improve the activities of the compounds varying the substitution on the triazole nucleus. Certain 1,2,4-triazole derivatives are of interests due to their bioactivity, including antibacterial (3-5) and antifungal (6, 7) properties. In recent years, attention has been increasingly paid to the synthesis of bis-heterocycle compounds, which exhibit various biological activities (8-11). Keeping these observations in mind and in continuation of our work on the synthesis of heterocyclic compounds containing nitrogen and sulfur (12, 13) and bis-heterocyclic compounds (14-17) with expected biological activity we report herein the synthesis of the versatile and hitherto unreported bis-thiosemicarbazides and their utility as a building blocks in the synthesis of several new bis-heterocyclic compounds. The 1,2,4 triazole nucleus has recently been incorporated into a wide variety of therapeutically interesting drugs candidates including H_1/H_2 histamine receptor blockers, fungicidal (18), anti-depressant (19) and plant growth regulator (20). The 1,3,4-thiadiazoles are also associated with pharmacological activities viz. diuretic (21) and antiinflammatory (22).

Ultrasound has increasingly been used in organic synthesis. Sonochemistry is becoming more and more important for a variety of synthetic organic reactions utilizing ultrasound as an energy source to generate radicals and initiate the electron transfer processes. It has numerous applications in medicine science and it can dramatically affect the rate of chemical reaction and yield of the product. Various types of sonochemical reactions have been reported such as azole (23), Reformatsky reaction (24), oxidation of substances like hydroquinones (25), pinacol coupling (26) etc.

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EXPERIMENTAL

Melting points of all synthesized compounds were determined in open capillaries in an electrothermal apparatus and are uncorrected. The purity of the compounds was monitored by thin layer chromatography on silica gel coated aluminium plates (Merck) using UV light as visualizing agent. The IR spectra (KBr, in cm^{-1}) were recorded on Perkin-Elmer spectrophotometer in the range of 4000-400 cm^{-1}. The ^1H NMR spectra were recorded on Varian 500 MHz NMR spectrophotometer using $CDCl_3$/DMSO-d_6 as a solvent and TMS as an internal standard (chemical shifts in δ ppm). The mass spectra were taken on a Jeol sx-102/PA-6000 (EI) spectrometer. C, H, N determinations were run on Carlo Erba 1108 (CHN) Elemental Analyzer. Experiments under ultrasound irradiation were carried out in sonicator manufactured by Dakshin.

Acid hydrazide of 5, 5-dimethylcyclohexane-1, 3-dione (2)

Method A (ultrasound method):

5,5-Dimethylcyclohexane-1,3-dione **(1)** (1.04 g, 0.01 mole), semicarbazide hydrochoride (2.22 g, 0.02 mole), sodium acetate (3.2 g, 0.04 mole) in absolute alcohol (10 mL) were placed in round bottom flask and subjected to ultrasound irradiation for 7 min. Progress of reaction was monitored by TLC. After completion of reaction, the content was dumped in crushed ice and filtered. The product was recrystallized from ethanol to yield **2.** Yield 68 %, m.p. 225°C.

Method B (conventional method)

The same amounts of reagents in round bottom flask were refluxed on water bath for 3 h. The reaction was monitored by TLC and after completion of the reaction the content was poured onto crushed ice. The solid obtained was filtered off, washed with water and recrystallized from ethanol to give compound **2**. Yield 58%, m.p. 225°C.

1,3-Bis-imino-[1-(carboxy)-4-substituted phenylthiosemicarbazide]-5,5-dimethylcyclohexane (3a-f)

Method A (ultrasound method):

Substituted isothiocyanate (0.02 mole), **2** (2.54 g, 0.01 mole) and ethanol (15 mL) were exposed to ultrasound irradiation for 12 min. Upon completion of the reaction (monitoring by TLC) the mixture was quenched onto crushed ice. The product that precipitated out was filtered, washed with water and recrystallized from glacial acetic acid. The physical data of the compounds are given in Table 1.

Method B (conventional method)

The same amounts of reagents were refluxed on water bath for 4 h. The reaction was monitored by TLC and after completion of the reaction, the contents were poured onto crushed ice. The solid obtained was filtered off, washed with water and recrystallized from glacial acetic acid to yield compounds **3a-f**.

3 (a) IR (cm^{-1}): 3222 (NH), 1676 (C=O), 1593 (C=N), ^1H NMR, DMSO-d_6 (δ, ppm): 0.92 (s, 6H, 2×CH_3), 1.32 (s, 2H, CH_2), 2.3 (s, 4H, 2×CH_2), 7.1-7.65 (m, 10H, ArH), 9.4 (s, 2H, NH), 11.1(s, 1H, NH).

3(f) IR(cm^{-1}): 3200 (NH),1620 (C=O), 1550 (C=N), ^1H NMR, DMSO-d_6(δ, ppm): 0.9 (s, 6H, 2×CH_3), 1.31 (s, 2H, CH_2), 2.15 (s, 4H, 2×CH_2), 3.9 (s, 3H, OCH_3), 7.05-7.35 (m, 8H, ArH), 9.3 (s, 2H, NH) 10.9 (s, 1H, NH).

1,3-bis-imino-[5-(substituted) phenylamino-1,3,4-thiadiazol-2-yl-]-5,5-dimethylcyclohexane (4)

Method A (ultrasound method):

A mixture of **3** (0.005 mole) and conc. H_2SO_4 (5 mL) was subjected to ultrasound irradiation for 30 min.The reaction mixture was poured onto crushed ice. The solid separated was filtered, washed with water and recrystallized from ethanol. The physical data of the compounds are given in Table 1.

Method B (conventional method):

The same amounts of reagents were stirred at 0°C for 1 h, then allowed to stand at room temperature for 2.5 h and then poured onto ice. The product was isolated in a similar manner as described above.

4(a) IR (cm^{-1}): 3378 (NH), 1670 (C=O), 1456 (C-S-C), ^1H NMR, $CDCl_3$ (δ, ppm): 1.4 (s, 6H, 2×CH_3), 1.72 (s, 2H, CH_2), 2.2 (s, 4H, 2×CH_2), 7.1-7.40 (m, 10H, ArH), 8.45 (s, 1H, NH).^{13}C NMR (ppm) 27 (2×CH_3), 32.45 (2×CH_2), 68.96 (CH_2), 121-137 (aromatic carbon), 162 (C=N), 172 (C=N), 188.74 (C=N), MS (m/z): 488.

4(f) IR (cm^{-1}): 3378 (NH), 1670 (C=O), 1456 (C-S-C). ^1H NMR $CDCl_3$ (δ, ppm): 1.45 (s, 6H, 2×CH_3), 1.75 (s, 2H, CH_2), 2.4 (s, 4H, 2×CH_2), 3.9 (s, 3H, OCH_3), 6.87-7.72 (m, 8H, ArH), 8.3 (s,1H, NH).

1,3-bis-imino-[4-(substituted) phenyl-5-mercapto-1,2,4-triazol-3-yl-]-5,5-dimethylcyclohexane (5)

Method A (ultrasound method):

A mixture of **3** (0.005 mole) and 2 M NaOH (10 mL) was subjected to ultrasound irradiation for

Table 1. Characterization of the synthesized compounds

Compd. no.	R_1	R_2	Molecular formula*	Molecular weight	Melting point (°C)	Yield (%)	
						Ultrasound	Conv.
3a	H	H	$C_{24}H_{22}N_8O_2S_2$	518.65	200	82	58
3b	CH_3	H	$C_{26}H_{32}N_8O_2S_2$	552.65	210	86	54
3c	H	CH_3	$C_{26}H_{32}N_8O_2S_2$	552.65	218	80	57
3d	Cl	H	$C_{24}H_{26}N_8O_2CI_2S_2$	593.56	215	87	59
3e	H	Cl	$C_{24}H_{26}N_8O_2CI_2S_2$	593.56	205	84	62
3f	OCH_3	H	$C_{26}H_{32}N_8O_4S_2$	584.72	198	81	59
4a	H	H	$C_{24}H_{24}N_8S_2$	488.64	70	72	58
4b	CH_3	H	$C_{26}H_{32}N_8S_2$	520.73	90	78	68
4c	H	CH_3	$C_{26}H_{32}N_8S_2$	520.73	96	72	64
4d	Cl	H	$C_{24}H_{26}N_8Cl_2S_2$	561.56	73	79	67
4e	H	Cl	$C_{24}H_{26}N_8Cl_2S_2$	561.56	85	72	63
4f	OCH_3	H	$C_{26}H_{32}N_8O_2S_2$	552.72	95	75	65
5a	H	H	$C_{24}H_{24}N_8S_2$	488.64	165	77	60
5b	CH_3	H	$C_{26}H_{32}N_8S_2$	520.73	168	79	62
5c	H	CH_3	$C_{26}H_{32}N_8S_2$	520.73	165	77	60
5d	Cl	H	$C_{24}H_{26}N_8Cl_2S_2$	561.56	148	70	58
5e	H	Cl	$C_{24}H_{26}N_8Cl_2S_2$	561.56	152	71	56
5f	OCH_3	H	$C_{26}H_{32}N_8O_2S_2$	552.72	158	76	55

* All the compounds gave satisfactory elemental analysis

Table 2. Antibacterial activity of compounds **4** and **5**

Compound no.	Concentration (µg/mL)	Zone of inhibition (in mm)*				
		Gram Positive			Gram Negative	
		S. aureus	*S. cerevesiae*	*C. diphtheriae*	*E. coli*	*P. aeruginosa*
4a	100	16	26	24	-	-
	200	18	22	23	08	09
4b	100	15	24	20	-	-
	200	18	23	22	07	09
4d	100	16	21	16	-	-
	200	18	23	18	11	09
4e	100	24	22	21	-	-
	200	23	18	20	08	08
5a	100	20	21	21	-	-
	200	22	24	20	08	09
5b	100	24	20	18	-	-
	200	22	21	20	08	08
5d	100	20	19	16	-	-
	200	24	20	18	10	11
5e	100	18	20	21	-	-
	200	22	18	24	08	10
Ampicillin trihydrate	50	26	23	28	24	21
DMSO	—	0	0	0	0	0

* Diameter of the hole was 6 mm

30 min. After completion of the reaction (TLC monitoring), the mixture was poured into ice-cold water. The solid obtained was filtered off, washed with diluted HCl followed by water and recrystallized from glacial acetic acid. The physical data of the compounds are given in Table 1.

Method B (conventional method):

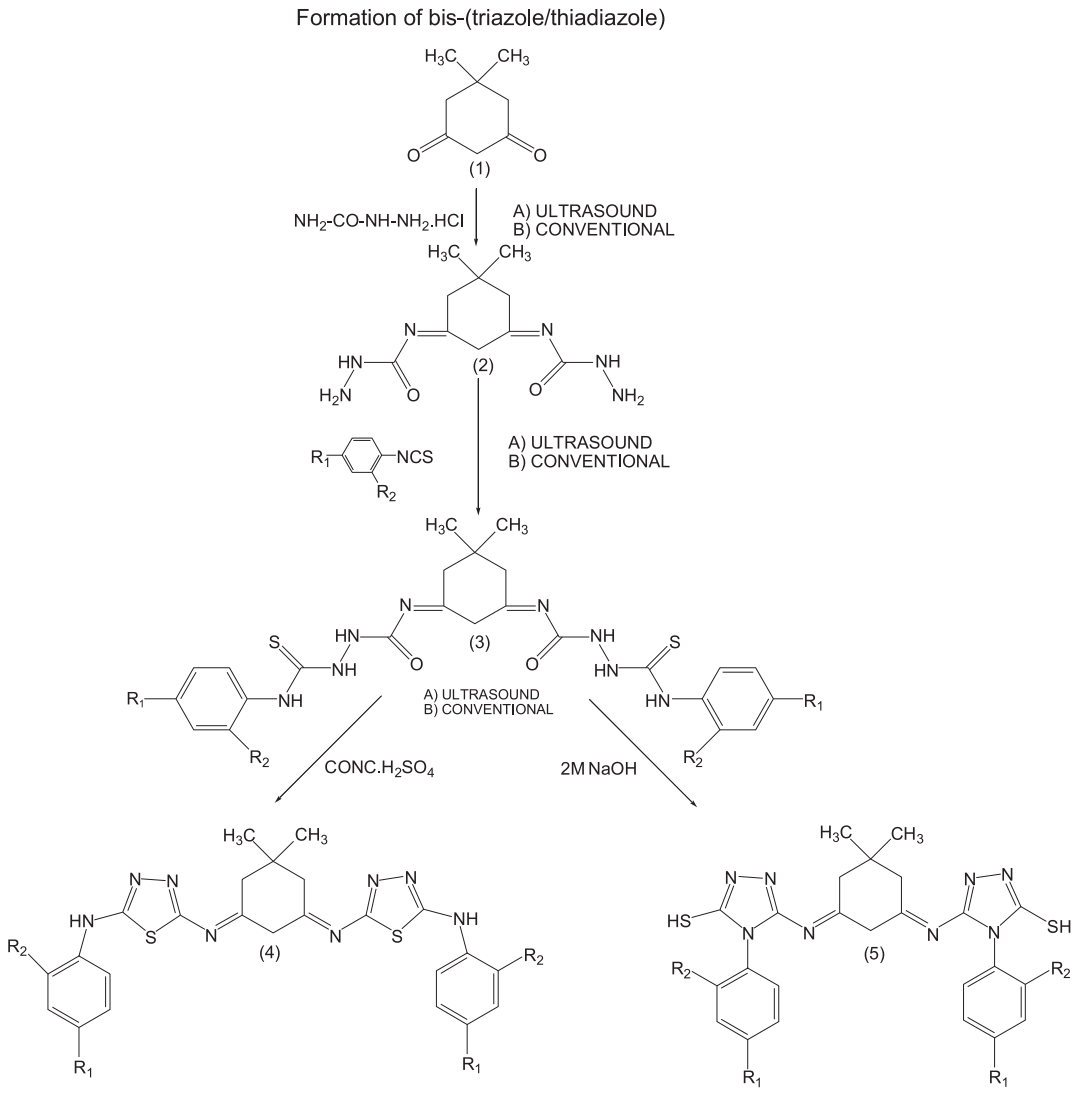
A mixture of **3** (0.005 mole) and 2 M NaOH (10 mL) was heated under mild condition for 4.5 h. The product was isolated in a similar manner as described above.

5 (a) IR (cm^{-1}): 3255 (NH), 1408 (C-S-C), 1515 (C=N), ^{1}H NMR (δ, ppm): 1.20 (s, 6H, 2×CH_3), 1.60 (s, 2H, CH_2), 2.15 (s, 4H, 2×CH_2), 7.05-7.35 (m, 10H, ArH), 10.5 (s, 1H, SH). MS (m/z): 489.

5 (f) IR (cm^{-1}): 3100 (NH), 1450 (C-S-C), 1515 (C=N), ^{1}H NMR (δ, ppm): 1.15 (s, 6H, 2×CH_3),1.32 (s, 2H, CH_2), 2.38 (s, 4H, CH_2), 3.85 (s, 3H, OCH_3), 6.6-7.45 (m, 8H, ArH), 10.5 (s, 1H, SH).

RESULTS AND DISCUSSION

The new series of heterocyclic compounds **4** and **5** have been synthesized as depicted in Scheme 1**.** Compound **1** was treated with semicarbazide to give acid hydrazide **2** which on further treatment with substituted phenyl isothiocyanates gave 1,3-bis-



Scheme 1.

imino-[1-(carboxy)-4-substituted phenylthiosemicarbazide]-5,5-dimethylcyclohexane **3**. The structure assignments of compounds **3** were established by spectroscopic and elemental analysis. In its ^{1}H NMR spectral data the signal of the thiosemicarbazide group protons NHCS (10.5-11.5 ppm) and CONH (9.0-9.4 ppm) are observed. Also in the IR spectra the absorption of thiosemicarbazide NH (3210-3230 cm^{-1}), C=O (1650-1680 cm^{-1}) and C=N (1550-1580 cm^{-1}) clearly confirms the formation of **3.**

The bis-thiosemicarbazides **3** on treatment with conc. H_2SO_4 underwent cyclization and gave 1,3-bis-imino-[5-(substituted)-phenylamino-1,3,4-thiadiazol-2-yl-]-5,5-dimethylcyclohexanes **4.** The spectral data are in good agreement with the proposed structures. Thus, the IR spectra of compounds **4** showed NH band in the region of 3350-3400 cm^{-1} and C-S-C absorption bands at 1350-1450 cm^{-1}. Also their ^{1}H NMR spectra supported the formation of **4** by showing the N-H signal at 8.3-8.5 ppm. Similarly,1,3-bis-imino[-4-(substituted)phenyl-5-mercapto-1,2,4-triazol-3-yl-]-5,5-dimethylcyclohexanes **5** were obtained by boiling **3** with diluted NaOH. The confirmation of compounds **5** were due to the S-H group signal shown in the region between 10.4-10.6 ppm in ^{1}H NMR spectra supporting the proposed structure. (see Experimental section)

ANTIBACTERIAL ACTIVITY

All the newly synthesized compounds were initially screened for their *in vitro* antibacterial activities against the Gram-positive (*S. aureus, C. diphtheriae and S. cerevisiae*) and the Gram-negative (*E. coli and P. aeruginosa*) bacteria by disc diffusion. method (27). The compounds were tested at a concentration of 100 µg/mL and 200 µg/mL. The zone of inhibition was measured in millimeters and was compared with the reference standard antibiotic namely ampicillin trihydrate (50 µg/mL). The compounds tested displayed good activity toward the Gram-positive bacteria, but were less active against Gram-negative bacteria. The results of antibacterial screening studies are reported in Table 2.

CONCLUSION

In conclusion, the ultrasound irradiation for synthesis of the title compounds offers reduction in the reaction time, operation simplicity, cleaner reaction, easy work-up and improved yields. The procedure clearly highlights the advantages of ultrasound. The synthesized compounds 1,3-bis-imino-[5-(substituted) phenylamino-1,3,4-thiadiazol-2-yl-]-5,5-dimethylcyclohexane and 1,3-bis-imino-[4-(substituted) phenyl-5-mercapto-1,2,4-triazol-3-yl-]-5,5-dimethylcyclohexane derivatives showed promising antibacterial activity against Gram-positive bacteria *S. aureus, C. diphtheriae and S. cerevisiae*. The data reported in this article may be 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 and to the Head, Department of Microbiology for antimicrobial studies. Authors are also thankful to the Director, Institute of Science, Mumbai (India) for providing spectral analyses.

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Received: 9. 12. 2007