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Synthesis and antimicrobial activity of some novel 2-azetidinones and 4-thiazolidinones derivatives

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ABSTRACT

Several 2-azetidinones 2a-e and 4-thiazolidinones 3a-e have been synthesized from halo-substituted Schiff bases using conventional as well as microwave technique. The newly synthesized compounds were established on the basis of spectroscopic technique. Further, all compounds screened for antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus*. Most of the titled compounds show potent activity.

1. Introduction

Literature survey reveals that most of the compounds having thiazolidiones and azetidinones nucleus possess pharmacological action [1,2]. Azetidinones, which are part of antibiotics structures are known to exhibit interesting biological activities. A large number of 3-chloro monocyclic β-lactam possesses powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant and antitubercular activities [3-5]. They also function as enzyme inhibitors and are effective on the central nervous system [6-8]. 4-Thiazolidinones and its derivatives are known to possess a verity of physiological *viz*. analgesic local [9] and spiral [10] anesthetic, central nervous system (CNS) stimulant [11], hypnotics [12], antibacterial [13], antifungal [14], antitubercular [15] and antioxidant [16].

The classical synthesis of these compounds involves cycloaddition of monochloroacetyl chloride with imine (Schiff base) resulting in formation of 2-azetidinone (β-lactam) [17]. Conventional synthesis of 4-thiazolidinones involves the cyclocondensation reaction between Schiff base and mercaptoacetic acid [18]. The one pot and convenient synthesis of 4-thiazolidinones achieved by the reaction of enaminones with ethyl 2-bromo propionate [19]. As part of our interest towards the development of novel heterocycles [20-24], herein we wish to report the synthesis of 2-azetidinones **2a-e** and 4-thiazolidinones **3a-e** by the reaction of imines **1a-e** with chloroacetyl chloride and thioglycolic acid respectively using conventional as well as microwave technique (Scheme 1).

2. Experimental

2.1. Instrumentation

Melting points were determined in an open capillary tube and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer spectrometer. ^{1}H NMR spectra were recorded on a Gemini 300 MHz instrument in DMSO-d_6 as solvent and TMS as an internal standard. The mass spectra were recorded on Shimadzu GC/MS spectrometer. Elemental analyses were performed on a Perkin-Elmer 240 CHN elemental analyzer. A multimode LG domestic microwave (640 Watt) oven induced reactions were carried out in an open borosil glass vessel under atmospheric pressure.

2.2. Synthesis of Schiff bases (1a-e)

Compound **1a-e** is synthesized according to given method in literature [25]. Equimolar quantities of halogeno substituted benzaldehyde and substituted aromatic amines were dissolved in methanol (15 mL) acetic acid (0.5 mL) was added and refluxed for 2 hr. After completion of reaction (monitored on TLC), the reaction mixture was cooled and poured in water, solid separated out. Solid was filtered, washed with water and crystallized from ethanol to give corresponding Schiff bases **1a-e**.

2.3. General procedure for preparation of 2-azetidinones (2a-e)

2.3.1. Conventional technique

A solution of 2-[(2,6-dichlorophenylimino)-methyl]3-bromo-4,5-dimethoxybenzene (0.001 mole, 0.463 mg) in dry dioxane (15 mL) was added to well stirred mixture of chloroacetyl chloride (0.002 mole) and triethyl amine (0.003 mole) in dry dioxane at 0-5 ^{o}C. The reaction mixture was stirred for 6 hr. Excess of solvent was distilled. The resultant solid was poured into ice-cold water. The separated solid was filtered and recrystallized from alcohol to give **2e**.

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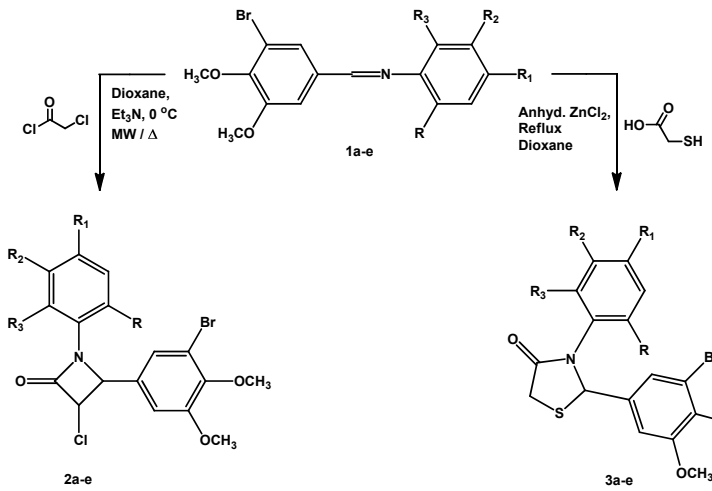

Scheme 1

Table 1. Physical and analytical data 2-azetidinones derivatives.

Entry	R	R_1	R_2	R_3	M.p., °C	Yield, %	
						Conventional technique	Microwave technique
2a	I	H	I	NO_2	158	68	84
2b	I	H	I	Cl	215	74	90
2c	Cl	I	H	Cl	162	75	89
2d	H	NO_2	H	I	180	70	86
2e	Cl	H	H	Cl	178	72	88

2.3.2. Microwave technique

A mixture of 2-[(2,6-dichlorophenylimino)-methyl]3-bromo-4,5-dimethoxybenzene (0.001 mole, 0.463 mg) in dry dioxane (15 mL) was taken in conical flask, and chloroacetyl chloride (0.002 mole) and triethyl amine (0.003 mole) were added slowly at 0-5 °C. The reaction mixture was irritated in a microwave oven for 7 min with short interval of 20 sec. to avoid the excessive evaporation of solvent. The separated solid was filtered and recrystallized from ethyl alcohol to give **2e**. Some of the physical data of synthesized compounds **2a**-**e** are given in Table 1.

4-(3-Bromo-4,5-dimethoxy-phenyl)-3-chloro-1-(3,6-diiodo-2-nitro-phenyl)-azetidin-2-one (**2a**): FT-IR (KBr, ν, cm^{-1}): 2937 (Arom. C-H str.), 1672 (C=O str.), 1468, 1455 (Arom. C=C str.), 1387 (C-N str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.87 (s, 1H, -CH), 3.83 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 5.03 (s, 1H, CH-Cl), 7.27-8.08 (m, 4H, ArH). MS (EI, *m/z* (%)): 693.5 (M^+, 80). Anal. calcd. for $C_{17}H_{12}O_5N_2I_2BrCl$: C, 29.41; H, 1.73. Found: C, 29.35; H, 1.75%.

4-(3-Bromo-4,5-dimethoxy-phenyl)-3-chloro-1-(2-chloro-3,6-diiodo-phenyl)-azetidin-2-one (**2b**): FT-IR (KBr, ν, cm^{-1}): 2934 (Arom. C-H str.), 1670 (C=O str.), 1460, 1438 (Arom. C=C str.), 1390 (C-N str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.85 (s, 1H, -CH), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.05 (s, 1H, CH-Cl), 7.35-8.12 (m, 4H, ArH). MS (EI, *m/z* (%)): 682 (M^+, 25). Anal. calcd. for $C_{17}H_{12}O_3I_2Cl_2NBr$: C, 29.91; H, 1.75. Found: C, 29.95; H, 1.73%.

4-(3-Bromo-4,5-dimethoxy-phenyl)-3-chloro-1-(2,6-dichloro-4-iodo-phenyl)-azetidin-2-one (**2c**): FT-IR (KBr, ν, cm^{-1}): 2937 (Arom. C-H str.), 1670 (C=O str.), 1482, 1442 (Arom. C=C str.), 1388 (C-N str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.85 (s, 1H, -CH), 3.86 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.07 (s, 1H, CH-Cl), 7.17-7.98 (m, 4H, ArH). MS (EI, *m/z* (%)): 590 (M^+, 40). Anal. calcd. for $C_{17}H_{12}O_3Cl_3IBrN$: C, 34.57; H, 2.03. Found: C, 34.52; H, 2.06%.

4-(3-Bromo-4,5-dimethoxy-phenyl)-3-chloro-1-(2-iodo-3-nitro-phenyl)-azetidin-2-one (**2d**): FT-IR (KBr, ν, cm^{-1}): 2943 (Arom. C-H str.), 1673 (C=O str.), 1473, 1432 (Arom. C=C str.), 1392 (C-N str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.87 (s, 1H, -CH), 3.89 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 5.02 (s, 1H, CH-Cl), 7.13-8.10 (m, 5H, ArH). MS (EI, *m/z* (%)): 567 (M^+, 40). Anal. calcd. for $C_{17}H_{13}O_5N_2IBrCl$: C, 35.97; H, 2.29. Found: C, 35.92; H, 2.32%.

3-Chloro-1-(2,6-dichlorophenyl)-4-(3-bromo-4,5-dimehtoxy phenyl)-2-azetidinone (**2e**): FT-IR (KBr, ν, cm^{-1}): 2924 (Arom. C-H str.), 1670 (C=O str.), 1470, 1450 (Arom. C=C str.), 1390 (C-N str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 2.85 (s, 1H, -CH), 3.81 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 5.0 (s, 1H, CH-Cl), 7.32-7.91 (m, 5H, ArH). MS (EI, *m/z* (%)): 464 (M^+, 60). Anal. calcd. for $C_{17}H_{13}O_3Cl_3BrN$: C, 43.96; H, 2.58. Found: C, 43.90; H, 2.60%.

2.4. General procedure for preparation of 4-thiazolidinone (3a-e)

2.4.1. Conventional technique

A mixture of 2-[(2,6-dichlorophenylimino)-methyl]3-bromo-4,5-dimethoxybenzene (0.001 mole, 0.462 mg) in dioxane (15 ml) containing anhydrous $ZnCl_2$ (0.01 g) and thioglycolic acid (0.001 mole) was refluxed for 8 hrs. The reaction mixture was cooled and poured into ice cold water. The separated solid was filtered and recrystallized from dioxane to give **3e**.

2.4.2. Microwave technique

A mixture of 2-[(2,6-dichlorophenylimino)-methyl]3-bromo-4,5-dimethoxybenzene (0.01 mole, 0.462 mg) in dioxane (15 mL) containing anhydrous $ZnCl_2$ (0.01 g) and thioglycolic acid (0.001 mole) was irradiated in a microwave oven for 8-10 min. with short interval of 20 sec. to avoid the excessive evaporation of solvent. The separated solid was filtered and recrystallized from ethyl alcohol to give **3e**. Some of the physical data of synthesized compounds **3a**-**e** are given in Table 2.

Table 2. Physical and analytical data 4-thiazolidinones derivatives.

Entry	R	R_1	R_2	R_3	M.p., °C	Yield, %	
						Conventional technique	Microwave technique
3a	I	H	I	NO_2	162	70	82
3b	I	H	I	Cl	178	65	88
3c	Cl	I	H	Cl	190	60	84
3d	H	NO_2	H	I	128	58	75
3e	Cl	H	H	Cl	149	72	90

2-(3-Bromo-4,5-dimethoxy-phenyl)-3-(3,6-diiodo-2-nitro-phenyl)-thiazolidin-4-one (**3a**): FT-IR (KBr, ν, cm^{-1}): 2860 (Arom. C-H str.), 1779 (C=O str.), 1578, 1522, 1443 (Arom. C=C str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.29 (s, 1H, CH), 3.74 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.91 (s, 2H CH_2S), 7.20-8.48 (m, 4H, ArH). MS (EI, *m/z* (%)): 691 (M^+, 72). Anal. calcd. for $C_{17}H_{13}O_5N_2I_2BrS$: C, 29.52; H, 1.88. Found: C, 29.57; H, 1.86%.

2-(3-Bromo-4,5-dimethoxy-phenyl)-3-(2-chloro-3,6-diiodo-phenyl)-thiazolidin-4-one (**3b**): FT-IR (KBr, ν, cm^{-1}): 2872 (Arom. C-H str.), 1782 (C=O str.), 1583, 1532, 1448 (Arom. C=C str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.27 (s, 1H, CH), 3.72 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.92 (s, 2H CH_2S), 7.08-8.30 (m, 4H, ArH). MS (EI, *m/z* (%)): 680.5 (M^+, 55). Anal. calcd. for $C_{17}H_{13}O_3I_2BrClSN$: C, 29.97; H, 1.91. Found: C, 29.92; H, 1.93%.

2-(3-Bromo-4,5-dimethoxy-phenyl)-3-(2,6-dichloro-4-iodo-phenyl)-thiazolidin-4-one (**3c**): FT-IR (KBr, ν, cm^{-1}): 2883 (Arom. C-H str.), 1780 (C=O str.), 1573, 1555, 1460 (Arom. C=C str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.28 (s, 1H, CH), 3.75 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.90 (s, 2H CH_2S), 7.11-8.20 (m, 4H, ArH). MS (EI, *m/z* (%)): 588 (M^+, 30). Anal. calcd. for $C_{17}H_{13}O_3Cl_2IBrSN$: C, 34.69; H, 2.21. Found: C, 34.65; H, 2.24%.

2-(3-Bromo-4,5-dimethoxy-phenyl)-3-(2-iodo-4-nitro-phenyl)-thiazolidin-4-one (**3d**): FT-IR (KBr, ν, cm^{-1}): 2887 (Arom. C-H str.), 1783 (C=O str.), 1578, 1527, 1458 (Arom. C=C str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.25 (s, 1H, CH), 3.73 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 4.88 (s, 2H CH_2S), 7.07-8.10 (m, 5H, ArH). MS (EI, *m/z* (%)): 565 (M^+, 75). Anal. calcd. for $C_{17}H_{14}O_5N_2IBrS$: C, 36.10; H, 2.47. Found: C, 36.14; H, 2.44%.

2-(3-Bromo-4,5-dimethoxy-phenyl)-3-(2,6-dichloro-phenyl)-thiazolidin-4-one (**3e**): FT-IR (KBr, ν, cm^{-1}): 2868 (C-H str.), 1776 (C=O str.), 1589, 1506, 1446 (Arom. C-H str.). ^{1}H NMR (300 MHz, DMSO-d_6, δ, ppm): 3.30 (s, 1H, CH), 3.72 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 4.94 (s, 2H, CH_2S), 7.26-8.52 (m, 5H, Ar-H). MS (EI, *m/z* (%)): 462 (M^+, 60). Anal. calcd. for $C_{17}H_{14}O_3Cl_2BrSN$: C, 44.15, H, 3.03. Found: C, 44.19; H, 3.07%.

2.5. Antimicrobial activity

The antibacterial activities of the synthesized compounds (**2a-e** and **3a-e**) were determined by agar well diffusion method [24,26]. The compounds were evaluated for antibacterial activity against *Bacillus subtilis* and *Escherichia coli*. The antifungal activity was assessed against *Aspergillus niger* and *Aspergillus flavus.* The antibiotic streptomycin (25 µg/mL) and fluconazole used as reference drug for antibacterial and antifungal activity, respectively. Dimethyl sulphoxide (1%, DMSO) used a control without compound.

The culture strains of bacteria were maintained on nutrient agar slant at 37 ± 0.5 °C for 24 h [24,26]. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 mL of respective bacterial culture strain suspension prepared in sterile saline (0.85%) of 105 CFU/mL dilutions. The wells of 6 mm diameter were filled with 0.1 mL of compound solution at fixed concentration 25 µg/mL separately for each bacterial strain. All plates were incubated at 37 ± 0.5 °C for 24 h. Zone of inhibition were noted in mm, Table 3.

For antifungal activity, all culture strains of fungi maintained on potato dextrose agar (PDA) slant at 27 ± 0.2 °C for 24-48 h, until sporulation [24,26]. Spore of strains were transferred into 5 mL of sterile distilled water containing 1% Tweenty-80 (to suspend the spore properly). The spores were counted by haemocytometer (106 CFU/mL). Sterile PDA plate was prepared containing 2% agar; 0.1 mL of each fungal spore suspension was spread on each plate and incubated at 27±0.2 °C for 12 h. After incubation well prepared using sterile cork borer and each agar well was filled with 0.1 mL of compound solution at fixed concentration 25 µg/mL. The plates were kept in refrigerator for 20 min for diffusion and then incubated at 27±0.2 °C for 7 days. After incubation, zone of inhibition were measured in mm along with standard, Table 3.

Table 3. Antimicrobial activity of 2-azetidinones and 4-thiazolidinones.

Entry	Zone of inhibition in mm			
	Bacillus subtilis	*Escherichia coli*	*Aspergillus niger*	*Aspergillus flavus*
2a	12	12	-	-
2b	24	18	20	18
2c	22	19	17	18
2d	11	-	12	09
2e	14	-	07	-
3a	12	16	11	13
3b	29	17	12	12
3c	25	26	19	24
3d	14	17	14	11
3e	18	15	14	13
Streptomycin	26	24	-	-
Fluconazole	-	-	25	27

3. Result and discussion

3.1. Synthesis

In view of the importance of this class of heterocycles and in continuation of our earlier investigation, reported the synthesis of 4-thiazolidinones from imines and some of the thiazolidinones were found to have antibacterial action [27]. Therefore in present paper, we synthesized new class of 2-azetidinones and 4-thiazolidinones by cyclocondensation reaction of imines **1a-e** (Scheme 1).

The starting iodoanilines required for the preparation of imines were prepared by iodination of substituted anilines using molecular iodine and iodic acid by refluxing technique [28]. Bromination of 3,4-dimethoxybenzaldehyde was carried out using Br_2/acetic acid as brominating agent to yield 3-bromo-4,5-dimethoxybenzaldehyde. The substituted iodo-anilines and 3-bromo-4,5-dimethoxybenzaldehyde on condensation in presence of slightly acidic medium to yield Schiff bases **1a-e** [25].

The compounds **1a-e** on cyclocondensation with chloroacetyl chloride affords 2-azetidinones, **2a-e**, and with thioglycolic acid affords 4-thiazolidinones, **3a-e**, using both conventional as well as microwave irradiation (MWI) technique. MWI technique were used over conventional technique due to the application of microwave (MW) irradiation as a nonconventional energy source for activation of reactions has now become a very popular and useful technology in organic chemistry [29]. Many researchers have described accelerated organic reactions towards proving the synthetic utility of MW irradiation in routine organic synthesis [30]. Thus MW technique has advantage including easy work-up procedure, short reaction time, and does not need effort for isolation of products giving high percentage yields. The structures of newly synthesized compounds **2a-e** and **3a-e** have been confirmed by elemental analysis, IR, ^{1}H NMR and MS spectral studies.

In ^{1}H NMR spectra of 2-azetidinones obtained at δ value 2.85 ppm and δ near 5.0 ppm is due to proton of CH-N and CH-Cl, respectively. The singlet of three proton of OCH_3 obtained near at δ value around 3.85 ppm. The ^{1}H NMR spectra of 4-thiazolidinones show characteristics δ value at 4.90 ppm due to two protons of -CH_2S. The δ value at 3.32 ppm is due to -CH of five-membered thiazolidinone ring.

3.2. Antimicrobial activity

The results of antimicrobial screening data are given in Table 3. In comparison with reference drugs, only compounds **3c** showed effective activity against all tested microbes. Compounds **2b** and **3b** showed near to par activity against *Bacillus subtilis.* The remaining compounds **2b**, **2c**, **3d** and **3e** displayed moderate antimicrobial activity against *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus*. On the other hand compound **2a**, **2d** and **2e** are inactive against *Aspergillus niger, Aspergillus flavus* and *Escherichia coli*, respectively. Results show that presence of halogen with methoxy substituent in basic 2-azetidinones and 4-thiazolidinone nucleus exhibits potent antimicrobial activity against various pathogens.

4. Conclusion

In summary, 2-azetidinones and 4-thiazolidinones derivative have been synthesized from halo-substituted imines using conventional as well as microwave technique. Percent yields of the products obtained by microwave technique are higher than conventional technique. Newly synthesized derivatives **2b**, **3b** and **3c** showed effective antimicrobial activity against tested microbes.

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