

BIOLOGICAL ACTIVITY OF SOME (2E)-SUBSTITUTED-2-ETHYLIDENE-5,6-DIPHENYLIMIDAZO[2,1-B][1,3]THIAZOL-3-(2H)-ONES

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ABSTRACT

Certain (2E)-substituted-2-ethylidene-5,6-diphenylimidazo[2,1-b][1,3]thiazol-3-(2H)-ones (4a-f) have been synthesized by the condensation of 4,5-diphenylimidazolin-2-thiones (3) with different aromatic aldehydes and chloroacetic acid in presence of acetic anhydride, anhydrous sodium acetate and glacial acetic acid. These compounds were characterized by their analytical and spectral data. The title compounds were found to be efficient antibacterial agents on evaluation.

Key words: Imidazothiazole, Biological Activity, Antibacterial Activity

INTRODUCTION

A number of imidazole and thiazole derivatives¹⁻⁴ have been reported to possess antibacterial activity. Certain other pharmacological activities⁵⁻⁸ have been reported. In other words the azole moiety is an important structural feature of many biologically active compounds. In view of such reports, we herein report the synthesis of some (2E)-substituted-2-ethylidene-5, 6-diphenylimidazo [2, 1-b][1,3]thiazol-3-(2H)-ones and antibacterial activity associated with them. The required 4, 5-diphenylimidazolin-2-thione (3) has been synthesized by the fusion of benzoin (1) with thiourea (2) in oil-bath. The 4,5-diphenylimidazolin-2-thione on condensation with chloroacetic acid and different aromatic aldehydes in presence of acetic anhydride, anhydrous sodium acetate and glacial acetic acid afforded synthesis of (2E)-substituted-2-ethylidene-5,6-diphenylimidazo[2,1-b][1,3]thiazol-3-(2H)-ones. The synthetic route is depicted in Scheme-1.

The structures of the compounds were supported by elemental and spectral data. The IR (KBr) spectra of all the compounds showed characteristic peaks in cm^{-1} at 3060-3030 (aryl C-H), 2956-2945 (aliphatic C-H), 1580-1500 (C=N) 1239-1225 (C-N) and 1598-1594 (C=CH). ¹H NMR spectra displayed signals showing proton singlet at δ : 6.9 for olefinic hydrogen atom (C=CH), multiplet at 7.3-7.9 (aromatic hydrogens) and corresponding peaks to confirm the structures.

EXPERIMENTAL

Melting points of the compounds were determined in open capillaries and are uncorrected. Purity of the compounds was checked by micro TLC using silica gel G coated glass plates using benzene-methanol (9:1, v/v) as irritant and iodine vapour as detecting agent. Elemental analyses for C, H and N were performed on a Perkin Elmer 240 C Elemental Analyzer and were with in 0.4 % of the theoretical values. The IR spectra (cm^{-1}) were recorded in KBr discs on a Perkin-

Elmer Infrared-283 spectrometer. ^1H NMR spectra were recorded in CDCl_3 solution at 200 MHz on a Bruker DPX spectrometer; chemical shifts (δ) are reported in ppm, with TMS as internal standard. All commercial chemicals were purchased from Nice and were used without further purification.

4, 5-Diphenylimidazolin-2-thione (3): 4,5-Diphenylimidazolin-2-thione (3) was prepared by mixing benzoin (1) (1.98 g, 0.01 mol) and thiourea (2) (0.76 g, 0.01 mol) and heated just over their mixed melting point, for three hr in an oil-bath. The reaction mixture was cooled and triturated with crushed ice (~150 g). The crude product separated was filtered, washed thoroughly with small portion of cold water and dried. The product was recrystallized from ethanol to get a pale yellow crystalline compound.

(2E)-Substituted-2-ethylidene-5,6-diphenylimidazo[2,1-b][1,3]thiazol-3-(2H)-ones (4a-f): The titled compounds, (2E)-Substituted-2-ethylidene-5,6-diphenylimidazo[2,1-b][1,3]thiazol-3-(2H)-ones (4a-f) were prepared by the following method. A mixture of 4,5-diphenylimidazolin-2-thione (3) (2.33 g, 0.01 mol) chloroacetic acid (0.95 g, 0.01 mol), fused sodium acetate (1.64 g, 0.02 mol) and aromatic aldehyde (0.01 mol) in acetic anhydride (15 ml) and glacial acetic acid (40 ml) were refluxed for 3 hr and cooled. The yellow colored solution was poured into cold water. The yellow solid then separated was filtered washed with water and recrystallized from glacial acetic acid and water to get white to pale yellow crystals.

4a: Yield 2.5g (68%), *m.p.* 226⁰, IR (KBr): 3063 (aromatic C-H stretching), 2956 (aliphatic C-H stretching), 1594 (-C=CH stretching), 1227 (C-N). ^1H NMR (CDCl_3) δ : 3.85 (9H, s, -OCH₃ groups), 6.90 (1H, s, -C=CH), 7.3-7.9 (12H, m, aromatic protons). MS *m/z*: 470 (M^+). Anal. Calcd for $\text{C}_{27}\text{H}_{22}\text{N}_2\text{O}_4\text{S}$: C, 68.92; H, 4.71; N, 5.95. Found; C, 68.76; H, 4.58; N, 5.79.

4b: Yield 2.1g (65%), *m.p.* 216⁰, IR (KBr): 3426 (-OH stretching), 3063 (aromatic C-H stretching), 1596 (-C=CH stretching), 1239 (C-N). ^1H NMR (CDCl_3) δ : 3.85 (3H, s, -OCH₃ groups), 4.61 (1H, s, -OH group), 6.90 (1H, s, -C-CH), 7.3-7.9 (13H, m, aromatic protons). MS *m/z*: 426 (M^+). Anal. Calcd for $\text{C}_{25}\text{H}_{18}\text{N}_2\text{O}_3\text{S}$: C, 70.41; H, 4.25; N, 6.57. Found; C, 70.26; H, 4.09; N, 6.43.

4c: Yield 1.82g (59%), *m.p.* 272⁰, IR (KBr): 3453 (-OH stretching), 3058 (aromatic C-H stretching), 2958 (aliphatic C-H stretching), 1597 (-C=CH stretching), 1235 (C-N). ^1H NMR (CDCl_3) δ : 3.85 (3H, s, -OCH₃ group), 4.45 (1H, s, -OH group), 6.90 (1H, s, -C-CH), 7.3-7.9 (14H, m, aromatic protons). MS *m/z*: 396 (M^+). Anal. Calcd for $\text{C}_{24}\text{H}_{16}\text{N}_2\text{O}_2\text{S}$: C, 72.71; H, 4.07; N, 7.07. Found; C, 72.86; H, 4.01; N, 7.18.

4d: Yield 2.0g (62%), *m.p.* 256⁰, IR (KBr): 3068 (aromatic C-H stretching), 1595 (-C=CH stretching), 1227 (C-N). ^1H NMR (CDCl_3) δ : 6.90 (1H, s, -C=CH), 7.3-7.9 (15H, m, aromatic protons). MS *m/z*: 380 (M^+). Anal. Calcd for $\text{C}_{24}\text{H}_{16}\text{N}_2\text{OS}$: C, 75.77; H, 4.24; N, 7.36. Found; C, 75.67; H, 4.16; N, 7.23.

4e: Yield 2.29g (63%), *m.p.* 252⁰, IR (KBr): 3063 (aromatic C-H stretching), 1594 (-C=CH stretching), 1492 (NO_2 asym), 1225 (C-N). ^1H NMR (CDCl_3) δ : 6.90 (1H, s, -C=CH), 7.3-7.9 (12H, m, aromatic protons). MS *m/z*: 425 (M^+). Anal. Calcd for $\text{C}_{24}\text{H}_{15}\text{N}_3\text{O}_3\text{S}$: C, 67.75; H, 3.55; N, 9.88. Found; C, 67.64; H, 3.42; N, 9.73.

4f: Yield 2.48g (62%), *m.p.* 224⁰, IR (KBr): 3063 (aromatic C-H stretching), 2956 (aliphatic C-H stretching), 1594 (-C=CH stretching), 1492 (NO_2 asym), 1227 (C-N). ^1H NMR (CDCl_3) δ : 6.90 (1H, s, -C=CH), 7.3-7.9 (12H, m, aromatic protons). MS *m/z*: 425 (M^+). Anal. Calcd for $\text{C}_{24}\text{H}_{15}\text{N}_3\text{O}_3\text{S}$: C, 67.75; H, 3.55; N, 9.88. Found; C, 67.66; H, 3.22; N, 9.71.

Antibacterial activity: The titled compounds were subjected to antibacterial screening by estimating the Minimum Inhibitory Concentration (MIC) by adopting two-fold serial dilution

technique⁹, using ampicillin as a positive control. DMF was used as solvent for all the compounds and as a control. The bacteria employed were *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Nutrient broth was used as growth medium for bacteriae. Nutrient broth was prepared by dissolving 13 g of dehydrated powder (HI-media) in 100 ml of distilled water. The media were sterilized by autoclaving at 15 lbs pressure for 20 minutes. Stock cultures were obtained by aseptically transferring a loopful of test organisms to 100 ml of sterile broth and incubated for 24 hours at 37°C. Stock cultures were standardized by placing in the incubator (37°C for bacteria) and shaken well. One ml of stock culture was aseptically transferred to 9 ml of sterile water containing 0.05% tween 80. This was mixed well using a cyclomixer and serially diluted from 10⁻¹ to 10⁻¹⁰. From each dilution, 0.2 ml was taken and spread on sterile nutrient agar plates for bacteria and incubated for 18 hr. After incubation, the numbers of colonies in the plate were counted. The number of colonies for a plate that was formed from the maximum diluted tube was noted. The number of microorganisms in stock were then calculated and expressed as colony forming units per ml (cfu/ml). By back calculation the stock culture was found to contain 15 x 10⁸ cfu/ml. Stock culture (0.1 ml) was diluted with nutrient broth (100 ml) to obtain 10⁵ cfu/ml. This was then used for further *in vitro* screening.

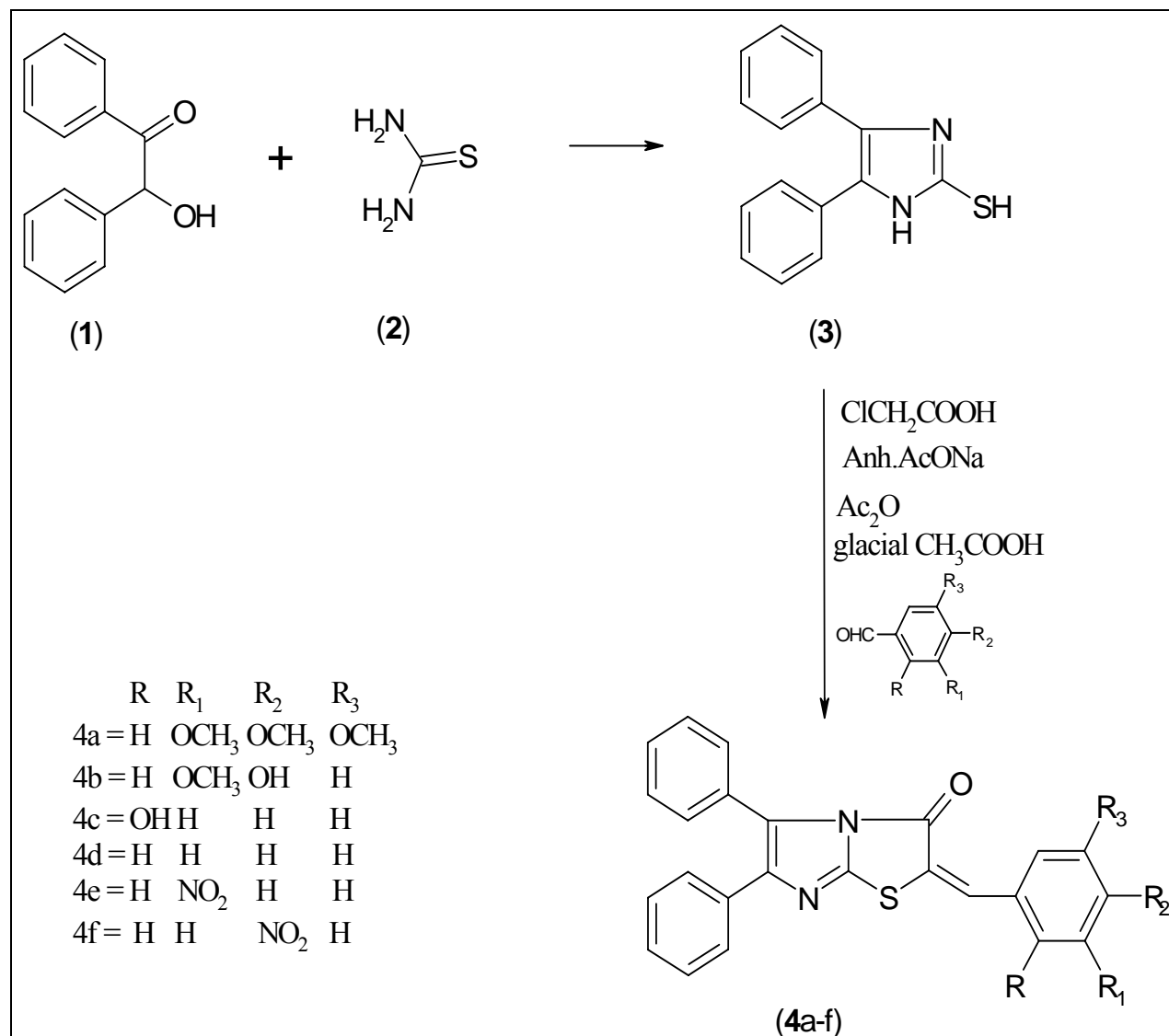
Solutions of the title compounds in DMF (1 mg/ml) were prepared and used for screening their antibacterial activity. The study involved a series of six assay tubes for each title compound against each microorganism. The entire test was done in duplicate. To the first assay tube, 1.8 ml of seeded broth and 0.2 ml of title compound (1 mg/ml) was added and mixed thoroughly and the two fold serial dilution was done up to the sixth tube containing 1 ml of seeded broth. The additions of the drug solution and serial dilution were done under strict aseptic conditions. Solvent control, negative control (growth control) and drug control were maintained during the experiment. The assay tubes were incubated at 37°C for 24 hours. The lowest concentration, which apparently caused complete inhibition of growth of microorganisms, was considered as the minimum inhibitory concentration (MIC). The results are presented in Table 2.

RESULTS AND DISCUSSION

It is interesting to note that many of the test compounds were effective against all the four strains of bacteria, however with a degree of variation. The MIC of compound 4a against *Bacillus subtilis* and *Escherichia coli* was 125 µg/ml and against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was 250 µg/ml. The MIC of compounds 4b and 4c against *Escherichia coli* was 125 µg/ml, against *Bacillus subtilis* was 250 µg/ml and against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was 500 µg/ml. The MIC of compound 4d against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was 250 µg/ml and against *Bacillus subtilis* was 500 µg/ml. The MIC of compounds 4e against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* was 250 µg/ml. The MIC of compound 4f against *Bacillus subtilis* and *Escherichia coli* was 250 µg/ml and against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was 500 µg/ml.

Antibacterial activity of all the compounds exhibited good activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* when compared to Ampicillin.

It can be concluded from antibacterial activity that substituted ethylidene group in imidazothiazole moiety the antibacterial activity is appreciable.



Scheme-1

TABLE-1: Physical and analytical data of title compounds (4a-f)

Comp.	R	R ₁	R ₂	R ₃	Yield (%)	M. P (°C)	% Analysis Found (Calcd)		
							C	H	N
4a	H	OMe	OMe	OMe	68	226	68.76 (68.92)	4.58 (4.71)	5.79 (5.95)
4b	H	OMe	OH	H	65	216	70.26 (70.40)	4.09 (4.25)	6.43 (6.57)
4c	OH	H	H	H	59	272	72.86 (72.71)	4.01 (4.09)	7.18 (7.07)
4d	H	H	H	H	62	256	75.67 (75.76)	4.16 (4.24)	7.23 (7.36)
4e	H	NO ₂	H	H	63	252	67.64 (67.75)	3.42 (3.55)	9.73 (9.88)
4f	H	H	NO ₂	H	62	224	67.66 (67.75)	3.22 (3.55)	9.71 (9.88)

IR (KBr) spectra of all the compounds showed characteristic peaks in cm⁻¹ for CH=C (1594-1598), C=N (1580-1500), C-N (1239-1225) and C=O (1726-1752).

TABLE-2: Antibacterial activity of compounds (4a-f)

Comp.	R	R ₁	R ₂	R ₃	Minimum Inhibitory Concentration (MIC)			
					<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
4a	H	OMe	OMe	OMe	125	250	125	250
4b	H	OMe	OH	H	250	500	125	500
4c	OH	H	H	H	250	500	125	500
4d	H	H	H	H	500	250	250	250
4e	H	NO ₂	H	H	250	250	250	250
4f	H	H	NO ₂	H	250	500	250	500

MIC in µg/ml. Antibacterial activities of all the synthesized compounds were tested against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

ACKNOWLEDGEMENTS

The authors are thankful to Hon'ble Dr. A. Shanmugasundaram, Chancellor, Vinayaka Mission's Research Foundation-Deemed University, Salem, India for providing facilities to carry out this work and to SPIC Science Foundation, Tuticorin for IR, ¹H NMR and elemental analysis.

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(Received: 3 October 2007

Accepted: 11 January 2008

RJC-130)

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