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Synthesis and bioactivity studies of 4-carbamoyl-1,2-disubstituted-1*H*-imidazole-5-carboxylic acid derivatives

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ABSTRACT

A series of new 4-carbamoyl-1,2-disubstituted-1H-imidazole-5-carboxylic acid derivatives have been prepared from the intermediate diethyl 2-mercapto-4,5-imidazole dicarboxylate. All the newly synthesized compounds were characterized by spectral studies and also tested for antibacterial, antifungal, antioxidant activity.

KEYWORDS

4-Carbamoyl-1,2-disubstituted-1H-imidazole-5-carboxylic acid;
Diethyl 2-mercapto-4,5-imidazole dicarboxylate;
Antibacterial;
Antifungal;
Antioxidant activity.

INTRODUCTION

Heterocyclic compounds play a vital role in synthetic organic and pharmaceutical chemistry. A good number of synthetic and naturally occurring heterocyclic systems find use in medicine, pesticides, agrochemicals, polymers paving the way for a considerable amount of research leading to new heterocyclic molecules having wide spread uses. The synthesis of nitrogen heterocyclic compounds has gained importance in recent years as they are present in vitamins, proteins, nucleic acids and other biologically important systems. Imidazoles are prominent nitrogen containing heterocyclic compounds that have many pharmacological properties and play important role in biochemical processes. Imidazole derivatives have attracted considerable attention due to their interesting biological activities. They have found to possess antibacterial[1], antifungal[2,3], anticancer[4], antiinflammatory[5], antitumor[6], antihelmintic[7], antiHIV[8], antihypertensive[9], antidepressant[10] activities. Because of these versatile applications different synthetic routes for substituted imidazoles have been developed.

A rapid and significant progress has been made in discovering and developing new imidazole derivatives which have potential medicinal applications. Owing to these observations, it was decided to prepare a series of new imidazole derivatives and evaluated for antimicrobial and antioxidant activity studies.

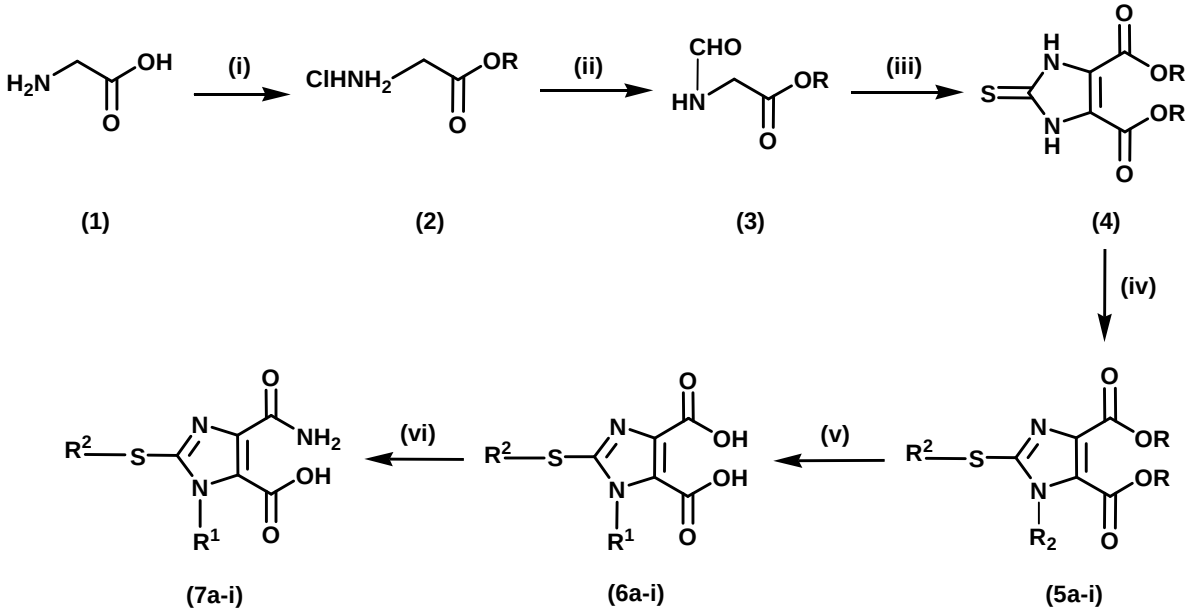
EXPERIMENTAL

All the reagents and solvents were obtained commercially and used without further purification. ^{1}H NMR spectra were recorded at 300 MHz with a Bruker Avance DPX 300 instrument. Mass spectra under electron impact conditions (EI) were recorded at 70 ev ionizing voltage with a VG Prospec instrument and presented as m/z (% rel.int). Melting points were determined over Thomas Hoover melting point apparatus and are uncorrected. Elemental analysis (C,

N, H) results were found to be in good agreement with the calculated values. TLC was used to monitor the progress of all the reactions and purity of the synthesized compounds.

General procedure

In continuation of our previous work[11], we herein report a simple approach for the synthesis of new 4-carbamoyl-1,2-disubstituted-1*H*-imidazole-5-carboxylic acid derivatives from the intermediate diethyl 2-mercapto-4,5-imidazole dicarboxylate starting from glycine from readily available reagents. The procedure adopted to obtain the target compounds are depicted in Scheme 1. Initially glycine ethylester hydrochloride **(2)** was prepared from glycine **(1)** on reaction with ethanol and thionyl chloride according to the literature report[12-14]. Compound **(2)** was treated with triethylamine and methyl formate to obtain *N*-formyl glycine ethylester **(3)**[15-18]. Compound **(3)** on treatment with diethyl oxalate and KSCN converted to intermediate product diethyl 2-mercapto-4,5-imidazole dicarboxylate **(4)**[19,20]. Compound **(4)** on reaction with alkyl or aryl halides in DMF and potassium carbonate at 0 °C afforded diethyl 1,2-disubstituted-1*H*-imidazole-4,5-dicarboxylate derivatives **(5)**. 1,2-Disubstituted-1*H*-imidazole-4,5-dicarboxylic acid derivatives **(6)** were obtained by stirring a mixture of compound **(5)**, ethanol and sodium hydroxide at room temperature for 5 hrs. To the compounds **(6)** (1 mol), acetic anhydride (5 ml) was added and the reaction mixture was heated. The resulting compound was dissolved in NH_3 (1.5 mol) solution and was stirred at room temperature for 1 hr. The progress of the reaction was monitored by TLC. After completion of the reaction, the solid obtained was collected, filtered and recrystallized from ethanol to obtain pure 4-carbamoyl-1,2-disubstituted-1*H*-imidazole-5-carboxylic acid derivatives **(7a-i)**.



Scheme 1

1-Benzyl-2-(benzylthio)-4-carbamoyl-1*H*-imidazole-5-carboxylic acid (7a)

Yield was found to be 78%, mp 170-172 °C, ^{1}H NMR spectrum ($CDCl_3$), δ ppm: 4.10 (s, 2H), 4.45 (s, 2H), 5.95 (s, 2H), 7.05-7.35 (m, 10H), 10.80 (s, 1H). Mass spectrum, m/z : 368 $(M+1)^+$. Found, %: C, 62.13; H, 4.68; N, 11.47. $C_{19}H_{17}N_3O_3S$. Calculated, %: C, 62.11; H, 4.66; N,11.44.

4-Carbamoyl-1-(2-methylbenzyl)-2-(2-methylbenzylthio)-1*H*-imidazole-5-carboxylic acid (7b)

Yield was found to be 76%, mp 204-205 °C, ^{1}H NMR spectrum ($CDCl_3$), δ ppm: 2.35 (s, 3H), 2.50 (s,3H) 4.12 (s, 2H), 4.48 (s, 2H), 5.95 (s, 2H), 7.05-7.35 (m, 8H), 11.0 (s, 1H). Mass spectrum, m/z : 396

$(M+1)^+$. Found, %: C, 63.81; H, 5.36; N, 10.64. $C_{21}H_{21}N_3O_3S$. Calculated, %: C, 63.78; H, 5.35; N, 10.63.

4-Carbamoyl-1-isobutyl-2-(2-methylbenzylthio)-1*H*-imidazole-5-carboxylic acid (7c)

Yield was found to be 75%, mp 250-251 °C, ^1H NMR spectrum ($CDCl_3$), δ ppm: 0.7 (d, 6H), 1.9 (m, 1H), 2.35 (s, 3H), 4.1(d, 2H), 4.4 (s, 2H), 5.95 (s, 2H), 7.05-7.35 (m, 4H), 10.98 (s, 1H). Mass spectrum, m/z : 348 $(M+1)^+$. Found, %: C, 58.79; H, 6.10; N, 12.11. $C_{17}H_{21}N_3O_3S$. Calculated, %: C, 58.77; H, 6.09; N, 12.09.

4-Carbamoyl-1-isobutyl-2-(isobutylthio)-1*H*-imidazole-5-carboxylic acid (7d)

Yield was found to be 78%, mp 180-182 °C, ^1H NMR spectrum ($CDCl_3$), δ ppm: 0.7 (d, 12H), 1.9 (m, 2H), 2.9 (d, 2H), 3.4 (d, 2H), 5.95 (s, 2H), 10.86 (s, 1H). Mass spectrum, m/z : 300 $(M+1)^+$. Found, %: C, 52.18; H, 7.09; N, 14.09. $C_{13}H_{21}N_3O_3S$. Calculated, %: C, 52.15; H, 7.07; N,14.04.

4-Carbamoyl-1-(4-methylbenzyl)-2-(4-methylbenzylthio)-1*H*-imidazole-5-carboxylic acid (7e)

Yield was found to be 78%, mp 195-196 °C, ^1H NMR spectrum ($CDCl_3$), δ ppm: 1.9 (s, 6H), 4.12 (s, 2H), 4.48 (s, 2H), 5.95 (s, 2H), 7.05-7.35 (m, 8H), 10.68 (s, 1H). Mass spectrum, m/z : 396 $(M+1)^+$. Found, %: C, 63.80; H, 5.36; N, 10.64. $C_{21}H_{21}N_3O_3S$. Calculated, %: C, 63.78; H, 5.35; N, 10.63.

4-Carbamoyl-1-(propyl)-2-(propylthio)-1*H*-imidazole-5-carboxylic acid (7f)

Yield was found to be 79%, mp 210-211 °C, ^1H NMR spectrum ($CDCl_3$), δ ppm: 0.8 (t, 6H), 1.45 (m, 4H), 2.8 (t, 2H), 3.62 (t, 2H), 5.95 (s, 2H) 10.98 (s, 1H). Mass spectrum, m/z : 272 $(M+1)^+$. Found, %: C, 48.72; H, 6.34; N, 15.52. $C_{11}H_{17}N_3O_3S$. Calculated, %: C, 48.69; H, 6.32; N, 15.49.

4-Carbamoyl-1-(isopropyl)-2-(isopropylthio)-1*H*-imidazole-5-carboxylic acid (7g)

Yield was found to be 78%, mp 163-164 °C, ^1H NMR spectrum ($CDCl_3$), δ ppm: 0.8 (d, 6H), 0.9 (d, 6H), 2.79 (m, 1H), 3.59 (m, 1H), 5.95 (s, 2H), 10.96 (s, 1H). Mass spectrum, m/z : 272 $(M+1)^+$. Found, %: C, 48.72; H, 6.33; N, 15.51. $C_{11}H_{17}N_3O_3S$. Calculated, %: C, 48.69; H, 6.32; N, 15.49.

4-Carbamoyl-1-(2-hydroxybenzyl)-2-(2-hydroxybenzylthio)-1*H*-imidazole-5-carboxylic acid (7h)

Yield was found to be 77%, mp 220-221 °C, ^1H NMR spectrum ($CDCl_3$), δ ppm: 4.13 (s, 2H), 4.65 (s, 2H), 5.20 (s, 1H), 5.95 (s, 2H), 7.05-7.35 (m, 8H), 10.88 (s, 1H). Mass spectrum, m/z : 400 $(M+1)^+$. Found, %: C, 57.15; H, 4.31; N, 10.54. $C_{19}H_{17}N_3O_5S$. Calculated, %: C, 57.13; H, 4.29; N, 10.52.

4-Carbamoyl-1-(4-hydroxybenzyl)-2-(4-hydroxybenzylthio)-1*H*-imidazole-5-carboxylic acid (7i)

Yield was found to be 77%, mp 236-237 °C, ^1H NMR spectrum ($CDCl_3$), δ ppm: 4.15 (s, 2H), 4.63 (s, 2H), 5.24 (s,1H), 5.95 (s, 2H), 7.05-7.35 (m, 8H), 10.90 (s, 1H). Mass spectrum, m/z : 400 $(M+1)^+$. Found, %: C, 57.15; H, 4.31; N, 10.54. $C_{19}H_{17}N_3O_5S$. Calculated, %: C, 57.13; H, 4.29; N, 10.52.

Antimicrobial testing

The compounds **(7a-i)** were tested for in vitro antimicrobial activity at two different concentrations 100 and 200 μg per disc. The antibacterial activity was screened against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria) and *Proteus vulgaris*, *Klebsiella pneumoniae* (Gram-negative bacteria) on nutrient agar plates at 37 °C for 24 hrs using chloramphenicol as reference drug. The compounds were also evaluated for antifungal activity against the fungi *Aspergillus niger* and *Pencillium chrysogenium* using Fluconazole as standard drug. Fungi cultures were grown on Potato dextrose agar (PDA) medium at 25 °C. The spore suspension was adjusted to 10^6 pores ml^{-1} at an mg ml^{-1} concentration by the Vincent and Vincent method[21].

Antioxidant testing

The compounds **(7a-i)** were tested for the antioxidant activity by nitric oxide and DPPH methods.

Assay for nitric oxide (NO) scavenging activity

Sodium nitroprusside (5 μM) in phosphate buffer pH 7.4 was incubated with 100 μM concentration of test compounds dissolved in a suitable solvent (methanol) and tubes were incubated at 25 °C for 120 min.

Control experiment was conducted with equal amount of solvent in an identical manner. At intervals 0.5 ml of incubation solution was taken and diluted with 0.5 ml of griess reagent (1% sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was read at 546 nm.

Reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (DPPH method)

The nitrogen centered stable free radical DPPH has often been used to characterize antioxidants. It is reversibly reduced and the odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm, which is purple in color. This property makes it suitable for spectrophotometric studies. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into 1,1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties. The solutions of test compounds (100 μM) were added to DPPH (100 μM) in ethanol. The tubes were kept at an ambient temperature for 25 minutes and the absorbance was measured at 517 nm. The difference between the test and the control experiments was taken and expressed as the percentage scavenging of the DPPH radical.

RESULTS AND DISCUSSION

The title compounds **(7a-i)** were synthesized from the intermediate diethyl 2-mercapto-4,5-imidazole dicarboxylate. The structure of the compounds is established by NMR and Mass spectra. The elemental analysis of **(7a)** indicated the MF of the compound to be $C_{19}H_{17}N_3O_3S$. Its ^1H NMR spectrum showed characteristic peaks at 4.10 (2H, s, S-CH_2), 4.45 (2H, s, N-CH_2), 5.95 (2H, s, $CONH_2$), 7.05-7.35 (m, Ar-10H), 10.80 (1H, s, COOH). The LCMS spectrum of **(7a)** at m/z: 368 $(M+1)^+$ indicating the structure of the compound to be 1-benzyl-2-(benzylthio)-4-carbamoyl-1*H*-imidazole-5-carboxylic acid. The other compounds **(7b-7i)** were synthesised using diferent substituents in R^1 and R^2 positions. (TABLE 1, Scheme 1).

TABLE 1 : 4-Carbamoyl-1,2-disubstituted-1*H*-imidazole-5-carboxylic acid derivatives (7a-i)

Compound	R^1	R^2
7a	$C_6H_5CH_2$-	$C_6H_5CH_2$-
7b	*o*-Me-C_6H_4-CH_2-	*o*-Me-C_6H_4-CH_2-
7c	$(CH_3)_2CHCH_2$-	*o*-Me-C_6H_4-CH_2
7d	$(CH_3)_2CHCH_2$-	$(CH_3)_2CHCH_2$-
7e	*p*-Me-C_6H_4-CH_2-	*p*-Me-C_6H_4-CH_2-
7f	$CH_3CH_2CH_2$-	$CH_3CH_2CH_2$-
7g	$(CH_3)_2CH$-	$(CH_3)_2CH$-
7h	*o*-OH-C_6H_4-CH_2-	*o*-OH-C_6H_4-CH_2-
7i	*p*-OH-C_6H_4-CH_2-	*p*-OH-C_6H_4-CH_2-

TABLE 2 : Antibacterial activity* of the target compounds (7a-i)

Compd.	Concentration (μg)	Zone of inhibition (mm)			
		Gram-positive bacteria		Gram-negative bacteria	
		S. aureus	*B. subtilis*	*P. vulgaris*	*K. pneumoniae*
7a	100	14	18	13	16
	200	17	20	15	20
7b	100	15	16	14	13
	200	17	18	17	17
7c	100	23	21	22	20
	200	26	25	23	22
7d	100	27	25	24	25
	200	30	29	28	28
7e	100	12	14	11	12
	200	15	16	13	14
7f	100	25	24	22	23
	200	28	29	25	27
7g	100	24	22	22	21
	200	26	28	24	23
7h	100	12	13	12	15
	200	15	17	14	17
7i	100	12	13	13	14
	200	15	17	16	16
Chloramphenicol	100	35	38	40	42
	200	39	41	44	45

***c = 100 μg / ml; *c = 200 μg / ml**

The preliminary antimicrobial testing results for the compounds **(7a-i)** are shown in TABLES 2 and 3. The results revealed that inhibitory activity against Gram-positive bacteria was higher than Gram-negative bacteria. The imidazole derivatives **(7c)**, **(7d)**, **(7f)** and **(7g)**

showed very good activity against Gram-positive bacteria and good activity against Gram-negative bacteria and remaining compounds were displayed least activity. All the test compounds **(7a)**, **(7b)**, **(7e)**, **(7h)** and **(7i)** showed good activity and compounds **(7c)**, **(7d)**, **(7f)** and **(7g)** exhibited least activity when compared to that of standard drug fluconazole at the same concentration as the test drugs against *Aspergillus niger and Pencillium chrysogenium*. The compounds **(7b)**, **(7c)**, **(7e)**, **(7f)**, **(7h)** and **(7i)** exhibited high antioxidant property in both nitric oxide and DPPH methods at 100 μM concentrations (TABLE 4).

TABLE 3 : Antifungal activity* of the target compounds (7a-i)

Compound	Concentration	Zone of Inhibition (mm)	
	(μg/ml)	*A.niger*	*P.chrysogenium*
7a	100	22	23
	200	26	25
7b	100	23	24
	200	26	28
7c	100	14	16
	200	18	20
7d	100	15	16
	200	18	20
7e	100	25	25
	200	29	28
7f	100	14	16
	200	21	20
7g	100	13	15
	200	16	19
7h	100	27	25
	200	30	30
7i	100	26	25
	200	28	29
Fluconazole	100	38	41
	200	42	44

***c = 100 μg / ml; *c = 200 μg / ml**

TABLE 4 : Antioxidant activity* of the target compounds (7a-i)

Compound	% Inhibition at 100 μM	
	Nitric oxide method	DPPH method
7a	32.75	35.25
7b	73.25	74.34
7c	70.15	71.82
7d	26.80	25.45
7e	72.62	73.25
7f	65.65	67.56
7g	28.80	27.62
7h	79.57	80.75
7i	77.74	77.62

***c = 100 μM**

CONCLUSION

The present finding in the present paper is a simple, efficient procedure for the synthesis of 4-carbamoyl-1,2-disubstituted-1*H*-imidazole-5-carboxylic acid derivatives using cheap and readily available reagents, easy work up procedure under mild reaction conditions. All the newly synthesized compounds **(7a-i)** were screened for antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* (Gram-positive bacteria) and *Proteus vulgaris*, *Klebsiella pneumoniae* (Gram-negative bacteria), antifungal activity against *Aspergillus niger* and *Pencillium chrysogenium*, antioxidant activity and some of the compounds found to exhibit significant biological activity.

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