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Research Article

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Synthesis, DNA protection and antimicrobial activity of some novel chloromethyl benzimidazole derivatives bearing dithiocarbamates

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ABSTRACT

Organic dithiocarbamates have received much attention due to their pivotal role in agriculture and their intriguing biological activities. They have also been used as protection groups in peptide synthesis, as linkers in solid-phase organic synthesis and recently in the synthesis of ionic liquids. Furthermore, dithiocarbamates are broadly employed in medicinal chemistry and have been used in cancer treatment. Hence, a series of chloromethyl benzimidazole derivatives bearing diverse dithiocarbamate moieties were designed and synthesized via three component reaction protocol. The synthesized dithiocarbamates were characterized by means of their IR , ^1H NMR , mass spectral data and elemental analysis. When these were evaluated for DNA protection and anti microbial activities some of them were found to possess significant activity.

Key words: Dithiocarbamates, Benzimidazole, Antimicrobial, DNA protection.

INTRODUCTION

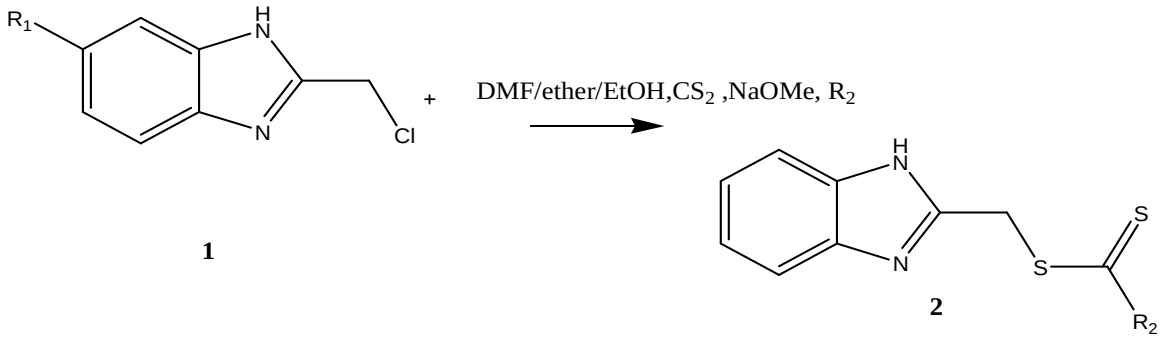
Dithiocarbamate (DTC) derivatives are well known as organic intermediates, rubber additive, additive of polluted water, vulcanizing agents and fungicides [1]. Dithiocarbamates have received considerable attention in recent times because of their occurrence in a variety of biologically active compounds [2]. They also play pivotal roles in agriculture, [3] and they act as linkers in solid-phase organic synthesis [4]. In addition, functionalized carbamates are an important class of compounds and their medicinal and biological properties warrant study [2]. They have also been used as protection groups in peptide synthesis, [5] as linkers in solid-phase organic synthesis [6] and recently in the synthesis of ionic liquids [7]. Furthermore, dithiocarbamates are broadly employed in medicinal chemistry and have been used in cancer treatment [8]. Therefore, the synthesis of dithiocarbamates has attracted a lot of attention recently. They have been used extensively as pharmaceuticals, [9] agrochemicals, [10] and intermediates in organic synthesis, [11]. DTC is a putative immunomodulator known as ditiocarb (or imuthiol) [12-15] and proposed to enhance immune responses in the treatment of AIDS[16-21]. Based on the above considerations, we proposed that benzimidazoles bearing DTC moiety should display some interesting antimicrobial and DNA protection activity. Therefore, we designed compounds with the aim to discover lead structure with DNA protection activity. Herein, we described the detailed synthetic route, screening results and structure-activity relationships of these designed compounds. Fortunately, some compounds with promising broad-spectrum DNA protection activity were identified.

EXPERIMENTAL SECTION

All the melting points reported in this series were determined in one end open capillaries using Thermonik Precision Melting Point cum Boiling Point Apparatus Model C-PMB-2, and are uncorrected. Purity of the compounds was confirmed by Thin layer chromatography using silica gel glass plates and a solvent system. The IR spectra were recorded using KBr Pellets on a Perkin-Elmer 1760 spectrophotometer (cm-1). ^{1}H NMR spectra were recorded on GE Omega 400

MHz spectrometer or Bruker Avance (300 MHz) spectrometer, using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a JEOL-JMS-D-300 spectrometer. All solvents are procured from Aldrich and Sigma and are used without further purification.

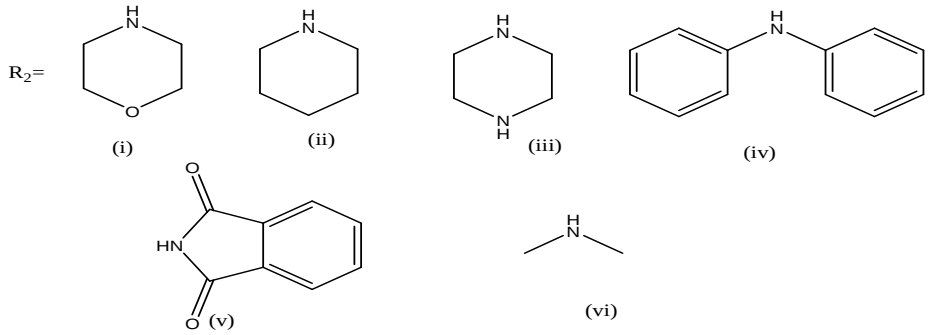
Scheme



1 **2**

(1*H*-benzo[*d*]imidazol-2-yl)methyl piperidine-1-carbodithioate

R_1=H, NO_2



Synthesis of 2-chloromethyl benzimidazole 1

Compound 1 is synthesized following the procedure reported earlier [22]

Synthesis of (1H-benzo[d]imidazol-2-yl)methyl pipiridine-1-carbodithioate 2

To a solution of amine (6 mmol) in DMF/ether/ethonal (4 mL) was added dropwise carbon disulfide (6 mmol) and anhydrous sodium methoxide (2 mmol). The resulted mixture was stirred at room temperature for 30 min. Then 2-chloromethyl benzimidazole **1** (3 mmol) was added by one-portion and stirring was continued. After completion of the reaction (monitored by TLC), the mixture was diluted with ice-coldwater (20 mL) and the precipitate was filtered, and recrystallized from ethanol to give the target compound **2(a-l)**.

Table 1. Physicochemical data of the compounds 2(a-l) and screening of solvents

Compounds	R_1	R_2	Formula[a]	(%) yield in different solvents			Melting Point (°C)
				Ethanol	Ether	DMF	
2a	-H	(i)	$C_{13}H_{15}N_3OS_2$	80	86	89	178
2b	-H	(ii)	$C_{14}H_{17}N_3S_2$	78	84	87	184
2c	-H	(iii)	$C_{13}H_{16}N_4S_2$	76	78	81	179
2d	-H	(iv)	$C_{21}H_{17}N_3S_2$	72	81	86	186
2e	-H	(v)	$C_{17}H_{11}N_3O_2S_2$	78	78	81	175
2f	-H	(vi)	$C_{11}H_{13}N_3S_2$	79	82	87	172
2g	$-NO_2$	(i)	$C_{13}H_{14}N_4O_3S_2$	71	75	79	189
2h	$-NO_2$	(ii)	$C_{14}H_{16}N_4O_2S_2$	74	77	80	189
2i	$-NO_2$	(iii)	$C_{15}H_{15}N_5O_2S_2$	79	81	85	185
2j	$-NO_2$	(iv)	$C_{21}H_{16}N_4O_2S_2$	81	86	89	183
2k	$-NO_2$	(v)	$C_{17}H_{10}N_4O_4S_2$	82	84	87	176
2l	$-NO_2$	(vi)	$C_{11}H_{12}N_4O_2S_2$	83	86	89	189
[a]Elemental analysis for C, H, N are within ± 0.5% of the theoretical values.							

Antibacterial activity

The newly synthesized compounds **2(a-l)** were tested for their *in vitro* antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* by using the agar disc diffusion method. The results of the preliminary antimicrobial testing of the prepared compounds, the typical broad spectrum of antibacterial drug ciprofloxacin are shown in Table 2. Among the tested compounds, four compounds showed considerable activity almost equal to the activity of ciprofloxacin. The other compounds were found to be moderate or least effective. In order to get some meaningful results, the structure-activity relationship was carried out. From the bacterial screening results it has been observed that the compounds having methoxy and nitro groups at 6-position showed moderate effect on the growth of bacteria.

Antifungal Activity

All the newly synthesized compounds **2(a-l)** were also screened for their antifungal activity against *Aspergillus niger*, *Aspergillus nodulans* and *Alternaria alternate* by food poison technique. The results of the preliminary antifungal testing of the prepared compounds, the typical broad spectrum of the potent antifungal drug amphotericin B are shown in Table 2. The antifungal activity data reveal that compounds containing methoxy and methyl substitutents at 6-position of quinoline ring, are showing excellent activity against the test fungi and nearly equal to the standard amphotericin B.

Table 2. Antimicrobial Activity of Compounds 2(a-l)

Growth inhibition zone diameter (mm)						
Compound	Antibacterial activity			Antifungal activity		
	S. aureus	*B. subtilis*	*E. coli*	*A. niger*	*A. nodulans*	*A. alternata*
2a	08	09	11	12	13	14
2b	10	11	14	---	13	11
2c	15	16	18	13	14	16
2d	16	18	17	19	21	18
2e	20	22	21	11	15	13
2f	14	16	15	19	17	15
2g	12	14	12	15	13	15
2h	19	21	20	20	21	19
2i	18	20	22	18	16	17
2j	15	16	13	09	11	12
2k	21	22	21	19	18	21
2l	09	11	13	11	13	14
Ciprofloxacin	22	22	25	---	---	---
Amphotericin B	---	---	---	20	23	20

The compounds **2(a-l)** and the standards used were of 100m g/8 mm discs.

DNA Protection Analysis

Sample stock was prepared in DMSO,Bacterial DNA was isolated from *Eschericha coli* was used for the assay. Various volumes of samples were added to 10µl DNA in a vial. Ferrous sulphate (10µl; 1mM) and Hydrogen

peroxide (10µl: 10mM) were added to the reaction vial. The mixture was incubated at 37˚C for 60 min. Agarose gel electrophoresis of this reaction mixture was carried out using 0.8% gel.

DNA protection activity of samples: Gel 1 – Samples 1-6

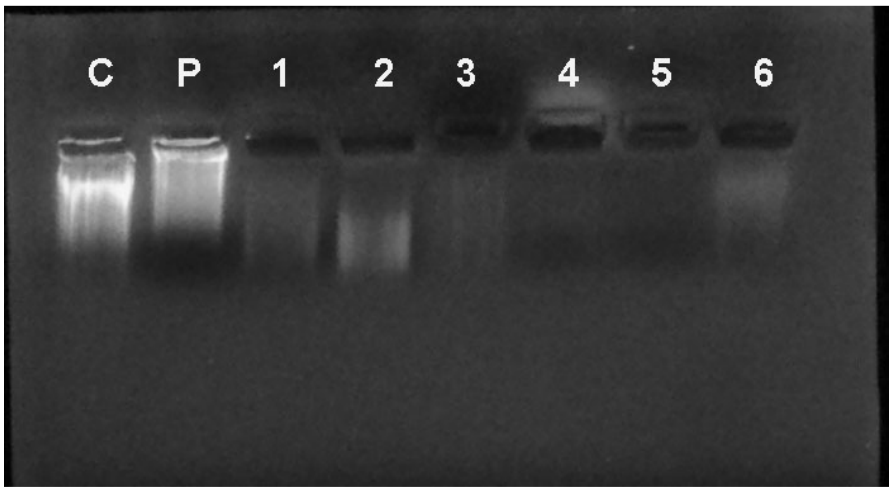


Table 3.1. DNA protection of Compounds 2(a-f)

DNA Protection		
Lane	**Sample and concentration tested**	**Results**
Lane Label C	Control DNA (untreated)	Clear band
Lane Label P	Positive control (Treated)	Smear
Lane Label a	Sample 1 -100µg	Smear -No protection
Lane Label b	Sample 2- 100µg	Faded nuclear band – partial protection
Lane Label c	Sample 3-100µg	Smear -No protection
Lane Label d	Sample 4-100µg	Faded nuclear band- partial protection
Lane Label e	Sample 5 -100µg	Smear -No protection
Lane Label f	Sample 6 -100µg	Faded nuclear band – partial protection

DNA protection activity of samples: Gel 2 – Samples 7-12

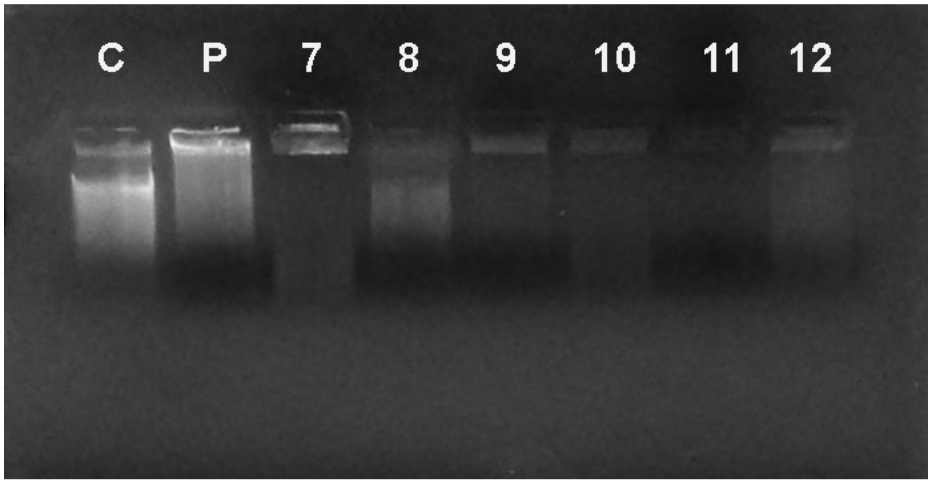


Table 3.2. DNA protection of Compounds 2(g-l)

DNA Protection		
Lane	**Sample and concentration tested**	**Results**
Lane Label C	Control DNA (untreated)	Clear band
Lane Label P	Positive control (Treated)	Smear
Lane Label g	Sample 7 -100µg	Faded nuclear band – partial protection
Lane Label h	Sample 8- 100µg	Faded nuclear band – partial protection
Lane Label i	Sample 9-100µg	Smear -No protection
Lane Label j	Sample 10-100µg	Smear -No protection
Lane Label k	Sample 11 -100µg	Smear -No protection
Lane Label l	Sample 12 -100µg	Smear -No protection

RESULTS AND DISCUSSION

Synthesis of title compounds by the earlier described method resulted in products with good yield. The final products were purified by the recrystallization techniques with methanol. The newly synthesized compounds **2(a-l)** were established on the basis of IR and 1H NMR spectroscopy method.

(1H-benzo[d]imidazol-2-yl)methyl morpholine-4-carbodithioate 2a:
3142(ArC-H), 1572(C=N), 1323(C-N), 1110(C=S), 1085(C-O-C of mor). ^{1}H NMR (400 MHz δ) 7.15-7.89 (m, 4H, Ar-H), 6.2 (s, 1H, NH, disappeared with D_2O), 3.11-3.97 (m, 8H, mor CH_2's), 4.46 (s, 2H, methyl CH_2).

(1H-benzo[d]imidazol-2-yl)methyl piperidine-1-carbodithioate 2b:
3146(ArC-H), 1574(C=N), 1312(C-N), 1124(C=S). ^{1}H NMR (400 MHz δ) 8.63-9.19 (m, 4H, Ar-H), 6.7 (s, 1H, NH, disappeared with D_2O), 4.11-4.73 (m, 10H, pip CH_2's), 5.16 (s, 2H, methyl CH_2).

(1H-benzo[d]imidazol-2-yl)methyl piperazine-1-carbodithioate 2c:
3215(ArC-H), 1539(C=N), 1275, 1298(C-N), 1214(C=S).). ^{1}H NMR (400 MHz δ) 8.14-8.97 (m, 4H, Ar-H), 6.3 (s, 1H, NH, disappeared with D_2O), 3.4(s, NH amine, disappeared with D_2O), 5.89-6.45 (m, 8H, piz CH_2's), 5.42 (s, 2H, methyl CH_2).

(1H-benzo[d]imidazol-2-yl)methyl diphenylcarbamodithioate 2d:
3219, 3228, 3267(ArC-H), 1598(C=N), 1265(C-N), 1210(C=S). ^{1}H NMR (400 MHz δ) 8.34-9.89 (m, 14H, Ar-H), 6.8 (s, 1H, NH, disappeared with D_2O), 5.82 (s, 2H, methyl CH_2).

(1H-benzo[d]imidazol-2-yl)methyl 1,3-dioxoisoindoline-2-carbodithioate 2e:
3255(ArC-H), 1524(C=N), 1232(C-N), 1672, 1690(C=O), 1124(C=S). ^{1}H NMR (400 MHz δ) 8.56-9.12 (m, 8H, Ar-H), 5.9 (s, 1H, NH, disappeared with D_2O), 4.42 (s, 2H, methyl CH_2).

(1H-benzo[d]imidazol-2-yl)methyl dimethylcarbamodithioate 2f:
3045(ArC-H), 1545(C=N), 1348(C-N), 1176(C=S). ^{1}H NMR (400 MHz δ) 8.21-8.72 (m, 4H, Ar-H), 6.5 (s, 1H, NH, disappeared with D_2O), 5.49 (s, 2H, methyl CH_2), 3.62 (s, 6H, CH_3).

(6-nitro-1H-benzo[d]imidazol-2-yl)methyl morpholine-4-carbodithioate 2g:
3282(ArC-H), 1652(C=N), 1357(C-N), 1201(C=S), 1125(C-O-C of mor). ^{1}H NMR (400 MHz δ) 8.15-9.14 (m, 4H, Ar-H), 6.2 (s, 1H, NH, disappeared with D_2O), 3.61-4.27 (m, 8H, mor CH_2's), 4.91 (s, 2H, methyl CH_2).

(6-nitro-1H-benzo[d]imidazol-2-yl)methyl piperidine-1-carbodithioate 2h:
3312(ArC-H), 1590(C=N), 1283(C-N), 1145(C=S). ^{1}H NMR (400 MHz δ) 8.34-9.49 (m, 4H, Ar-H), 6.3 (s, 1H, NH, disappeared with D_2O), 4.32-4.65 (m, 10H, pip CH_2's), 5.66 (s, 2H, methyl CH_2).

(6-nitro-1H-benzo[d]imidazol-2-yl)methyl piperazine-1-carbodithioate 2i:
3148(ArC-H), 1562(C=N), 1383(C-N), 1139(C=S). ^{1}H NMR (400 MHz δ) 8.76-9.37 (m, 4H, Ar-H), 6.43 (s, 1H, NH, disappeared with D_2O), 3.6 (s, NH amine, disappeared with D_2O), 5.43-6.15 (m, 8H, piz CH_2's), 5.42 (s, 2H, methyl CH_2).

(6-nitro-1H-benzo[d]imidazol-2-yl)methyl diphenylcarbamodithioate 2j:
3283, 3245, 3169(ArC-H), 1545(C=N), 1378(C-N), 1235(C=S).). ^{1}H NMR (400 MHz δ) 8.78-9.59 (m, 14H, Ar-H), 6.8 (s, 1H, NH, disappeared with D_2O), 5.52 (s, 2H, methyl CH_2).

(6-nitro-1H-benzo[d]imidazol-2-yl)methyl 1,3-dioxoisoindoline-2-carbodithioate 2k:
3215(ArC-H), 1595(C=N), 1690, 1685(C=O), 1268(C-N), 1232(C=S). ^{1}H NMR (400 MHz δ) 8.76-9.32 (m, 8H, Ar-H), 6.1 (s, 1H, NH, disappeared with D_2O), 4.76 (s, 2H, methyl CH_2).

(6-nitro-1H-benzo[d]imidazol-2-yl)methyl dimethylcarbamodithioate 2l:
3267(ArC-H), 1497(C=N), 1225(C-N), 1219(C=S). ^{1}H NMR (400 MHz δ) 8.67-9.15 (m, 4H, Ar-H), 6.2 (s, 1H, NH, disappeared with D_2O), 5.83 (s, 2H, methyl CH_2), 3.61 (s, 6H, CH_3).

In case of physico-chemical properties, screening of solvents were done to know the better yield of compounds. Compared to ethanol and ether DMF gave the best yield among the three solvents. In case of antimicrobial activity of the resulting compounds synthesized, compounds with pipirizine and pthalamide moeity showed good anti-bacterial results where as compounds with di methyl amine and di phenyl amine showed better anti-fungal activity **(Table 2)**. In case of DNA protection, compounds 2b,2d,2f,2g,2h showed faded nuclear bands in the gel **(Table 3.1 and Table 3.2)**.

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