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Research Article

SYMMETRICAL COUPLING OF 2-MERCAPTO BENZIMIDAZOLE DERIVATIVES AND THEIR ANTI-MICROBIAL ACTIVITY

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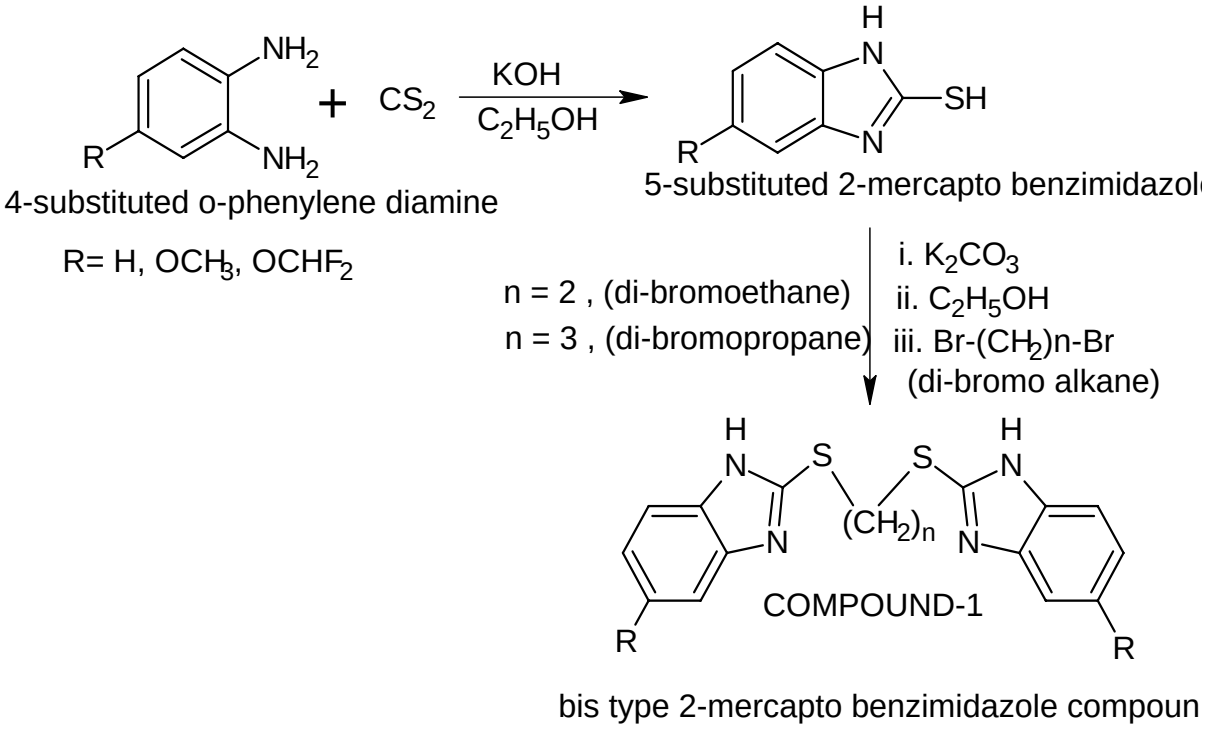
ABSTRACT

The conventional methodology was adopted to synthesize the titled compounds. The syntheses of titled compounds from starting compounds i.e 5-substituted 2-mercapto benzimidazoles were prepared from 4-substituted ortho-phenylene diamine and carbon disulfide in presence of KOH in single step. The Synthesized 5-substituted 2-mercapto benzimidazoles are symmetrically coupled by linking agents with two splitting off groups like 1,2 –dibromo ethane and 1,3-dibromo propane in ethanol using potassium carbonate as deacidifying agent, to get their respective substituted bis type 2-mercapto benzimidazole derivatives. The synthesized all benzimidazole derivatives were screened for anti bacterial activity using DMF as a solvent against the organisms, S.aureus and E.coli. The derivatives are also screened for Antifungal activity using Candida albicans by disc diffusion method on nutrient agar media. The standard drug used was Ampicillin for anti-bacterial and Ketoconazole for anti-fungal activity.

Keywords: Mercapto benzimidazole derivatives, Antimicrobial activity

INTRODUCTION

A number of benzimidazoline 2-thiones have been synthesized by the general method described by Van allan and Deacon.[1] 2-mercapto-benzimidazole which contain a hydrogen atom attached to nitrogen in the 1-position readily tautamerised.[2-3] N-acetyl benzimidazole has been prepared by heating 2-benzimidazole carboxylic acid with acetic anhydride decarboxylation occurs and forms the product. The majority of data on benzimidazoline 2-thione relates to S-alkylation and closely related processes are the synthesis of 2-thiocyanatobenzimidazoles from the reaction of benzimidazoline 2-thione with cynogen chloride or bromide and 2-benzimidazolyl thiocarbamates from addition of the 2-thione to aryl isocyanates.[4-5] Other routine procedures are the oxidation of 2-thiones to bis benzimidazolyl disulfides and benzimidazole 2-sulfonic acids by hydrogen peroxide. The reviews clearly emphasize the importance of Heterocycles in naturally occurring as well as synthetic agents and does an important class itself possess diversified pharmacological actions such as antimicrobial, antiprotozoal, antimalarial, and antiallergic etc.[6-8] This point encouraged further investigation in the field. The present work was formulated, bearing in mind that the biological activities of known moieties and attempting certain structural modification or adaptation in light of the recent trends in drug research incorporating newly emerged pharmacophores on existing moiety. Hence in the present study we synthesized some novel bis type 2-mercapto benzimidazoles and are screened for anti-bacterial, anti-fungal activities.



MATERIAL AND METHODS

Experimental

Melting points were determined by using Precision melting point apparatus in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel G plates using n-Hexane, ethyl acetate (6:4) and methanol: chloroform (1:9) solvent system and Ultraviolet lamp and iodine chambers used as a visualizing agent. IR-spectra were recorded using KBr pellets on a SHEMADZU 8000 series spectro-photometer. ^{1}H-NMR spectra on BRUKER 400 MHz Spectrophotometer using Ethanol as solvent and TMS as internal standard (chemical shift values expressed in ppm).

Procedure

Step-1[9]

1. Preparation of 2-mercapto benzimidazole. (Compound-I).

A mixture of 10.8gm (0.1mole) of o-phenylenediamine, 5.65 gm (0.1mole) of potassium hydroxide and 7.67 gm (0.1mole, 6.19ml) of carbon disulfide, 100ml of 95% ethanol and 15 ml of water in a 500ml round bottom flask heated under reflux for three hours. Then added 1-1.5 gm of charcoal cautiously and the mixture is further heated at the reflux for 10 minutes, the charcoal is removed by filtration. The filtrate is heated to 60-70^{o}C, 100ml of warm water is added, and acidified with dilute acetic acid with vigorous stirring. The product separated as glistening white crystals, and the mixture is placed in a refrigerator for three hours to complete the crystallization. The product is collected and dried over night at 40^{o}c. The dried product is recrystallised with ethanol.

2. Preparation of 5-methoxy 2-mercapto benzimidazole. (Compound-II)

15.2gm (yield 84) of 5-methoxy 2-mercapto benzimidazole was obtained from 13.8gm (0.1mole) of 4-methoxy o-phenylene diamine, 5.65 gm (0.1mole) of potassium hydroxide and 7.67 gm (0.1mole, 6.19ml) of carbon disulfide in the same manner as in (1).

3. Preparation of 5-difloromethoxy 2-mercapto benzimidazole. (Compound-III)

17 gm(78%) of 5-difloromethoxy 2-mercapto benzimidazole was obtained from 17.4 gm (0.1mole) of 4-difloromethoxy o-phenylene diamine, 5.65 gm (0.1mole) of potassium hydroxide and 7.67 gm (0.1mole, 6.19ml) of carbon disulfide in the same manner as in (1).

Step-2[10-11]

General procedure for the preparation of bis type benzimidazoles

Compound-a

5gm (0.026mole) of compound-I and 2.3 gm (0.013mole) of 1, 2 dibromoethane were dissolved in 60ml of ethanol, and 5gm of potassium carbonate is added as a deacidifying agent. And the thus-obtained solution was refluxed under stirring on water bath for about 14hrs. After cooling, it was neutralized with 2N aqeous NaOH solution. Crystals thus formed were collected by filtration, and are Washed with hydrous ethanol and acetonitrile.

Compound-b

10.1gm (yield 91%) of the intended compound was obtained from 5gm(0.026mole) of compound-I and 2.6gm(0.013mole) of 1, 3-dibromopropane in the same manner as in (1).

Compound-c

8.9gm (yield 85%) of the intended compound was obtained from 5gm (0.02mole) of compound-II and 2.0 gm(0.011mole) of 1, 2-dibromoethane in the same manner as in (1).

Compound-d

9.3gm (yield 85%) of the intended compound was obtained from 5gm (0.02mole) of compound-II and 2.2gm (0.01mole) of 1, 3-dibromopropane in the same manner as in (1).

Compound-e

8.2gm (yield 78%) of the intended compound was obtained from 5gm (0.019mole) of compound-III and 1.74gm (0.009mole) of 1, 2-dibromoethane in the same manner as in (1).

Compound-f

8.8gm (yield 82%) of the intended compound was obtained from 5gm (0.019mole) of compound-III and 1.8gm (0.009mole) of 1, 3-dibromopropane in the same manner as in (1).

Anti-bacterial activity

All the compounds synthesized in the present investigation were screened for their anti-bacterial activity by Cup plate Method. Antibacterial activities were tested on nutrient medium against, Staphylococcus aureus, and Escherchia coli which are representative types of gram positive and gram negative organisms respectively. The antibacterial activity of the compounds was assessed by disc-diffusion method[12-13]. The extent diameter of inhibition after 24 hours was measured as the zone of inhibition in millimeters.

Anti-fungal activity

Broth double dilution method [14]

The broth double dilution method was used to evaluate the minimal inhibitory concentration (MIC) of the test compounds. The classical method yields accurate, precise and quantitative results for the amount of antimicrobial agent that is needed to inhibit growth of microorganism. In screening the test compounds were dissolved in DMF, so as to give 8000µg/ml which was then serially diluted. Ketoconazole used as standard, was dissolved in sterile DMSO.DMF, DMSO were also tested as control.

RESULTS AND DISCUSSION

From the literature survey it reveals that benzimidazoles and 2-mercapto benzimidazoles have been reported for number of pharmacological activities and some molecules have shown significant activities and some compounds shows moderate and good activities.[15-18] Here we have synthesized some novel benzimidazole analogues and screened them for their anti-bacterial and anti-fungal activities and the results are as follows.

Bis type 2-mercapto benzimidazole derivatives are synthesized from substituted 2-mercaptobenzimidazoles (compounds I, II &III). The physiochemical parameters of compounds I, II & III was shown in table no-1.

Spectral data of Substituted 2- mercapto Benzimidazole Derivatives.

2- mercapto benzimidazole. (Compound-I)

IR (KBr):NH(str): 3154,3116, Ar-CH(str): 2981C-N(str):1259,1357,C=C(str): 1513,1467,C-S(str): 601,660. EI ms: m/z: 151(M+1).

5- methoxy 2-mercapto benzimidazole. (Compound-II)

IR(KBr):NH(str):3304, 3466, Ar-CH(str): 3007, 3023, 3063, C-N(str):1265, 1335, C=C(str): 1468, 1499, C-S(str): 577, 628, C-H of CH3 : 2886, 2962. EI ms: m/z: 181(M+1).

5- difloromethoxy 2-mercapto benzimidazole. (Compound-III)

IR(KBr):NH(str):3851, Ar-CH(str): 2970, 3099, C-N(str): 1258, 1336, 1355, C=C (str): 1472, 1497, 1527, C-S (str): 616, 653, 690, C-F of CF2 :1121, 1181, 1258, 1336. EI ms: m/z: 217 (M+1).

Compounds a-f, are synthesized by symmetrical coupling of compounds I, II &III using dibromo alkanes. The physicochemical parameters of synthesized bis type 2-mercapto benzimidazole derivatives were shown in table no-2.

Table 1: Physicochemical parameters of substituted 2-mercapto benzimidazole derivatives

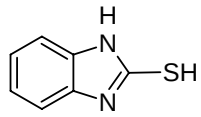
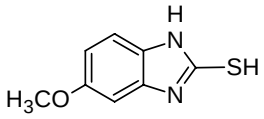
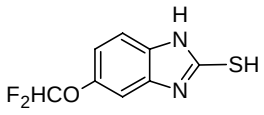
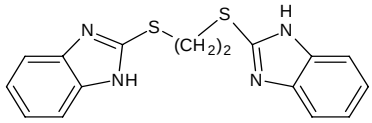
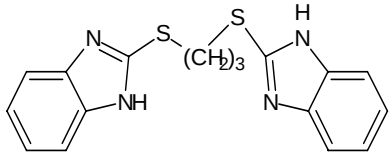
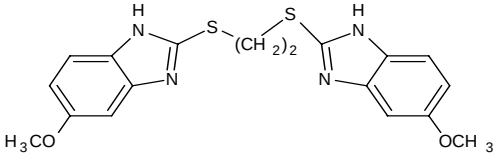
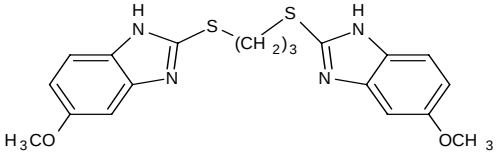
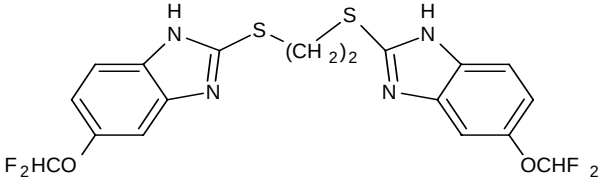
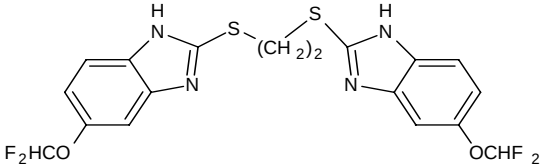
S.No	Compound code	Compound	Structure	Practical yield (gm)	Yield (%)	Melting point (°C)
1	I	2-mercapto benzimidazole		8.5	73	300-305
2	II	5-methoxy 2-mercapto benzimidazole		15.2	84	258-262
3	III	5-difloro methoxy 2-mercapto benzimidazole		17	78	255-256

Table 2: Physicochemical parameters of Bis type Benzimidazole derivatives

S.NO	Compound code	Structure	Yield (%)	Melting point (^{0}C)	Mol.Wt gm/mole
1	a		75	232-2350C	326
2	b		82	197-1990C	340
3	c		78	188-1900C	386
4	d		84	202-2050C	400
5	e		83	227-2290C	458
6	f		84	226-2310C	472

Spectral Data of Bis type Benzimidazole derivatives

1, 2-bis (2-mercaptobenzimidazole) ethane (Compound-a):

IR(KBr):NH(str):3386ArCH(str):3023,3052,CN(str):1270,1293,1346 ,1441C=C(str)1467,1592C-S(str):617,695C-H methylene(str): 2866. EI ms: m/z: 327(M+1), 349. H^1NMR (CDCl3,200MHZ): 7.21-7.62(m,8H, aromatic), 4.21(m,4H, methylene).

1, 3-bis (2-mercaptobenzimidazole) propane (Compound-b)

IR(KBr):NH(str):3382,ArCH(str):3061,3133,CN(str):1272,1298,135 2,C=C(str):1436,1470,1503,
1591,CS(str):617,661,CHmethylene(str):2880.EIms:m/z:341(M+1), 363.H^1NMR(CDCl3,200MHZ):6.94-7.42(m,8H, aromatic), 2.25-3.73(m,6H, methylene).

1, 2-bis (5-methoxy2-mercapto benzimidazole) ethane (Compound-c):

IR(KBr):NH(str):3304, Ar-CH(str):3004, 3025, 3061, C-N(str):1267, 1294, 1343, C=C(str):1433, 1460, 1499, 1596, C-S(str):627, 675, C-Hmethylene(str):2886, C-H methyl:2954. EI ms: m/z: 387(M+1), 409. H^1NMR (CDCl3,200MHZ): 7.12-7.41 (m,6H, aromatic) 3.87(s,6H,methyl, -O-),4.21(m,4H, methylene).

1, 3-bis (5-methoxy 2-mercapto benzimidazole) propane (Compound-d):

IR(KBr):NH(str):3348, Ar-CH(str):3068, C-N(str):1270, 1301, 1343, C=C(str):1449, 1489, 1595, C-S(str):624, C-Hmethylene(str):2831, 2934, C-Hmethyl:2884, 2982. EI ms: m/z: 401(M+1), 423, 221. H^1NMR (CDCl3,200MHZ): 7.12-7.26(m,6H,aromatic), 3.87(s,6H,methyl, -O-CH_3), 2.25-3.73 (m,6H, methylene).

1, 2-bis (5-difloromethoxy 2-mercapto benzimidazole) ethane. (Compound-e)

IR(KBr):NH(str):3548, Ar-CH(str):3098, C-N(str):1256, 1300, 1338, 1354, C=C(str):1471, 1498, 1527, 1557, C-S(str):615, 651, 687, C-H methylene (str):2871, C-F:1120, 1221, 1257, 1300. EI ms: m/z: 459(M+1), 242,243. H^1NMR (CDCl3,200MHZ):6.77-7.41 (m,6H, aromatic), 7.36(s,2H, -O-CHF2),4.21(m,4H, methylene).

1, 3-bis (5-difloromethoxy 2-mercapto benzimidazole) propane. (Compound-f)

IR(KBr):NH(str):3414, Ar-CH(str):3050,C-N(str):1269, 1297, 1347, C=C(str):1446, 1481, 1597,C-S (str): 618, 641, 678, C-H methylene (str): 2872, C-F: 1179, 1229, 1269, 1297. EI ms: m/z: 473(M+1), 495. H^1NMR (CDCl3,200MHZ):6.99-7.41 (m,6H, aromatic),7.36(s,2H, -O-CHF2),2.1-4.2(m,6H, methylene).

The synthesized all benzimidazole derivatives were screened for anti bacterial activity using DMF as a solvent against the organisms, S.aureus and E.coli by Cup plate method and were also screened for Antifungal activity using Candida albicans by disc diffusion method. The standard drug used was Ampicillin for antibacterial and Ketoconazole for antifungal activity.

The antimicrobial screening results reveals that compounds **a, b** exhibited poor activity at 50µg/ml, but at 100µg/ml they have shown moderate activity against S. aureus, and moderate activity against E. coli. The compounds **c, d,** have shown the moderate activity against E. Coli and S. Aureus at 50 µg/ml. but the same compounds at 100 µg/ml against same organism have shown very good activity. And **e, f** has shown the moderate activity against S. aureus at 100 µg/ml when compared with the standard drug Ampicillin.

The same Compounds also screened for the anti-fungal activity against Candida albicans the compounds **c, d** Showed highest degree of inhibition at 250µg/ml and 500µg/ml against C.albicans when compared with the standard drug Ketoconazole. However the activities shown by all the compounds tested were less than that of the standard.

The Discussion part mainly deals with the synthesized compounds against the antibacterial and anti fungal activity. The compounds **c, d,** have shown good anti bacterial activity due to the presence of electron donating group , **OCH_3** group which is attached at 5th position of the benzimidazole ring system and the compounds **e, f** may be due to the presence of **$OCHF_2$** group attached at the fifth position of the benzimidazole ring system.

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