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# Studies on Novel Azetidinone and Their Biological Studies

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**ABSTRACT**

2-(1H-benzo[d]imidazol-2-ylthio) acetohydrazide**(3)** undergoes facile condensation with aromatic aldehydes to afford the corresponding 2-(1H-benzo[d]imidazol-2-ylthio)-N-arylidene aceto hydrazide (**4a-f**) in good yields. Cyclocondensation of compounds (**4a-f**) with chloro acetyl chloride yields 2-(1H-benzo[d]imidazol-2-ylthio)-N-(3-chloro-2-(4-aryl)-4-oxoazetidin-1-yl)acetamide (**5a-f**). The structures of these compounds were established on the basis of analytical and spectral data. The synthesized compounds were tested for their antibacterial and antifungal activities.

**Keywords:** 2-(1H-benzo[d]imidazol-2-ylthio) acetohydrazide, azetidinone, Antibacterial activity and antifungal activity.

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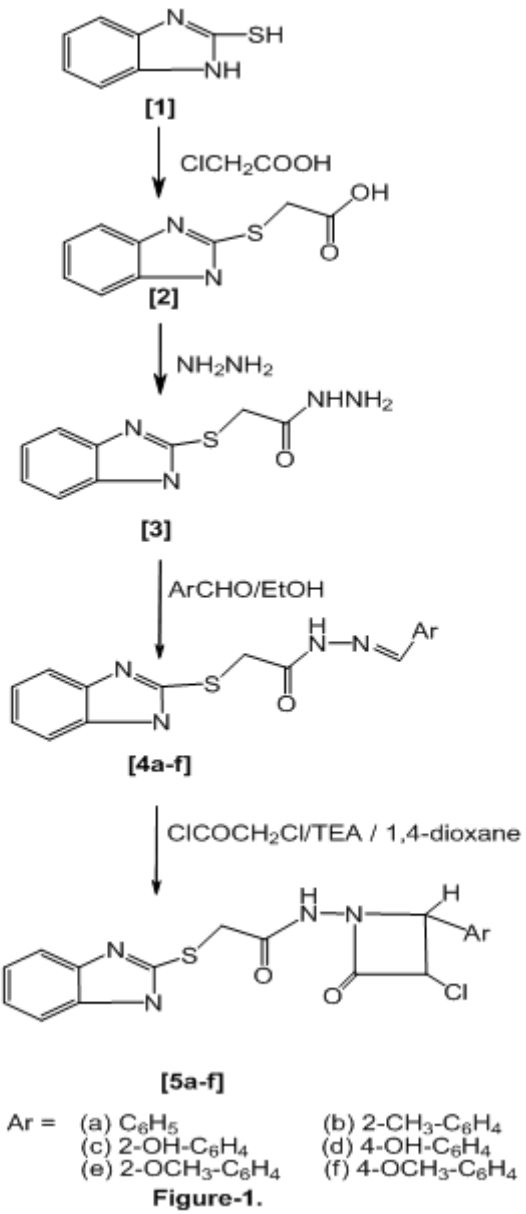
## INTRODUCTION

Heterocyclic compounds based on hydrazides exhibit diverse biological activities including antibacterial, antifungicidal, analgesic, anti-inflammatory properties [1-10]. These heterocyclic systems find wide use in medicine, agriculture and industry. One of the acetohydrazide and their condensed products play a vital role in medicinal chemistry[11-13]. A large number of azetidinones containing β-lactam rings [14-17] are known to exhibit various biological activities like antibacterial, antifungal [18] and antibiotic [19] activities. Hence, it was thought of interest in merging of both azetidinone and benzimidazole moieties may enhance the drug activity of compounds up to some extent or might posses some of the above mentioned biological activities. From this point of view, the objective of the present work is to prepare new derivatives of phthalimide containing an azetidinone moiety. Hence the present communication comprises the synthesis of 2-(1H-benzo[d]imidazol-2-ylthio)-N-(3-chloro-2-(4-aryl)-4-oxoazetidin-1-yl)acetamide**(5a-f)**. The research work is scanned in Figure-1.




Where, Ar = (a) $C_6H_5$ (b) 2-$CH_3$-$C_6H_4$ (c) 2-OH-$C_6H_4$ (d) 4-OH-$C_6H_4$ (e) 2-$OCH_3$-$C_6H_4$ (f) 4-$OCH_3$-$C_6H_4$

**Figure-1.**

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## EXPERIMENTAL

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer and $^{1}H$ NMR and $^{13}C$NMR spectra were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz and 100 MHz, respectively. LC-MS of selected samples taken on LC-MSD-Trap-SL_01046.

***Preparation of 2-(1H-benzo[d]imidazol-2-ylthio)- N'-arylidene aceto hydrazide (4a-f)***

***General procedure:–*** An equimolecular mixture of 2-(1H-benzo[d]imidazol-2-ylthio) acetohydrazide (3), (0.01mole) and the aromatic aldehydes (a-f) in ethanol (15ml) was refluxed on a water bath for 1.5-2 hrs. The solid separated was collected by filtration, dried and recrystallized from ethanol. The yields, melting points and other characterization data of these compounds are given in Table -1.

**Table: 1 Analytical Data and elemental analysis of compounds (4a-f)**

| Compd. | Molecular formula (Mol.wt.) | LC-MS Data | Yield | M.P.* $^{0}C$ | Elemental Analysis | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | %C | | % H | | %N | | %S | |
| | | | | | Found | Calcd. | Found | Calcd. | Found | Calcd. | Found | Calcd. |
| 4a | $C_{16}H_{14}N_4OS$ (310) | 313 | 87 | 241-243 | 61.90 | 61.92 | 4.52 | 4.55 | 18.04 | 18.05 | 10.31 | 10.33 |
| 4b | $C_{17}H_{16}N_4OS$ (324) | 344 | 83 | 236-238 | 62.92 | 62.94 | 4.95 | 4.97 | 17.26 | 17.27 | 9.86 | 9.88 |
| 4c | $C_{16}H_{14}N_4O_2S$ (326) | 329 | 82 | 238-240 | 58.85 | 58.88 | 4.30 | 4.32 | 17.15 | 17.17 | 9.80 | 9.82 |
| 4d | $C_{16}H_{14}N_4O_2S$ (326) | 328 | 80 | 239-241 | 58.86 | 58.88 | 4.31 | 4.32 | 17.16 | 17.17 | 9.81 | 9.82 |
| 4e | $C_{17}H_{16}N_4O_2S$ (340) | 344 | 83 | 236-238 | 59.96 | 59.98 | 4.72 | 4.74 | 16.44 | 16.46 | 9.40 | 9.42 |
| 4f | $C_{17}H_{16}N_4O_2S$ (340) | 344 | 83 | 236-238 | 59.96 | 59.98 | 4.72 | 4.74 | 16.44 | 16.46 | 9.40 | 9.42 |

***Preparation of 2-(1H-benzo[d]imidazol-2-ylthio)-N-(3-chloro-2-(4-aryl)-4-oxoazetidin-1-yl)acetamide (5a-f)***

***General procedure***

A mixture *2-(1H-benzo[d]imidazol-2-ylthio)- N'-arylidene aceto hydrazide* ***(4a-f)*** (0.002 mole) and triethyl amine (TEA) (0.004 mole) was dissolved in 1,4-dioxane (50 ml), cooled, and stirred. To this well-stirred cooled solution chloro acetyl chloride (0.004 mole) was added drop wise within a period of 30 minutes. The reaction mixture was then stirred for an additional 3 hours and left at room temperature for 48 hours. The resultant mixture was concentrated, cooled, poured into ice-cold water, and then air-dried. The product thus obtained was purified by column chromatography over silica gel using 35% ethyl acetate: 65% benzene as eluent. Recrystallization from ether/n-hexane gave white powered of 2-(1H-benzo[d]imidazol-2-ylthio)-N-(3-chloro-2-(4-aryl)-4-oxoazetidin-1-yl)acetamide(**5a-f**),which was obtained in 55-76% yield. The yields, melting points and other characterization data of these compounds are given in Table -2.

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**Table: 2 Analytical Data and elemental analysis of compounds (5a-f)**

| Compd. | Molecular formula (Mol.wt.) | LC-MS Data | Yield | M.P.* $^0$C | Elemental Analysis | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | %C | | % H | | %N | | %S | |
| | | | | | Found | Calcd. | Found | Calcd. | Found | Calcd. | Found | Calcd. |
| 5a | $C_{18}H_{15}N_4O_2SCl$ (386) | 402 | 55 | 221-223 | 55.86 | 55.88 | 3.90 | 3.91 | 14.46 | 14.48 | 8.28 | 8.29 |
| 5b | $C_{19}H_{17}N_4O_2SCl$ (400) | 421 | 62 | 233-235 | 56.91 | 56.93 | 4.25 | 4.27 | 13.97 | 13.98 | 7.98 | 8.00 |
| 5c | $C_{18}H_{15}N_4O_3SCl$ (402) | 428 | 76 | 236-238 | 53.66 | 53.67 | 3.73 | 3.75 | 13.90 | 13.91 | 7.95 | 7.96 |
| 5d | $C_{18}H_{15}N_4O_3SCl$ (402) | 426 | 72 | 237-239 | 53.65 | 53.67 | 3.73 | 3.75 | 13.89 | 13.91 | 7.94 | 7.96 |
| 5e | $C_{19}H_{17}N_4O_3SCl$ (416) | 434 | 68 | 232-234 | 54.72 | 54.74 | 4.10 | 4.11 | 13.42 | 13.44 | 7.68 | 7.69 |
| 5f | $C_{19}H_{17}N_4O_3SCl$ (416) | 435 | 64 | 231-232 | 54.73 | 54.74 | 4.09 | 4.11 | 13.43 | 13.44 | 7.68 | 7.69 |

## RESULTS AND DISCUSSION

It was observed that 2-(1H-benzo[d]imidazol-2-ylthio) acetohydrazide **(3)**, on condensation with aromatic aldehydes, yields 2-(1H-benzo[d]imidazol-2-ylthio)- N-arylidene aceto hydrazide **(4a-f)**. The structures of **(4a-f)** were confirmed by elemental analysis and IR spectra showing an absorption band at 1620-1640 (C=N), 3030-3080 $cm^{-1}$ (C-H, of Ar.), 1720-1750 $cm^{-1}$ (-CO), 2620-2560 $cm^{-1}$ (-CS), 2815-2850 $cm^{-1}$ ($-OCH_3$), 2950, 1370 $cm^{-1}$ ($-CH_3$). $^1$H NMR : 6.98 – 7.95 (10H, m) (Ar-H), 11.79-11.80 (1H, s) (-CONH), 8.43-8.80 (1H, s) (-N=CH),4b,1.56(3H,s)($-CH_3$); 4c,4d; 4.22-4.24 (1H,s) (-OH),4e,4f:3.65-3.69(3H,s)($-CH_3$). $^{13}$C NMR:117.8-118, 118.3-118.5, 121.6-122.1, 128.7-129.2, 129.3-129.5, 129.4-130.1, 131.3-131.5, 133.7-133.9, 133.9-134.2, 159.7-160.1(Ar-10C), 163.5-163.8 (-CONH), 146.9-150.4 (-CH);4b,14.8($CH_3$),4e,4f: 55.5-56.7 ($-OCH_3$). The C, H, N analysis data of all compounds are presented in Table -1.

The cyclocondensation of **(4a-f)** with chloro acetyl chloride resulted in formation of 2-(1H-benzo[d]imidazol-2-ylthio)-N-(3-chloro-2-(4-aryl)-4-oxoazetidin-1-yl)acetamide **(5a-f)**. The structures assigned to **(5a-f)** were supported by the elemental analysis and IR spectra showing absorption bands at 1750-1760 (C=O of monocyclic β-lactam),3035-3090$cm^{-1}$(C-H, of Ar.), 3450-3550 $cm^{-1}$ (-OH), 2820-2850 $cm^{-1}$ ($-OCH_3$), 2950, 1370 $cm^{-1}$ ($-CH_3$),1620(C=N ring),765(C-O-C ring). $^1$H NMR: 7.64 – 6.98 (8H, m) (Ar-H), 11.79-11.80 (1H, s) (-CONH), 7.8(1h,s)(-NH),5.19(1H,s)(N-$C_2$H),5.49(1H,s)($C_3$H),5b,1.56(3H,s)($-CH_3$); 5c,5d; 4.22-4.24 (1H,s)(-OH),5e,5f:3.65-3.69(3H,s)($-CH_3$).$^{13}$CNMR:114.3,114.3,115.6,115.6,123.2,123.2, 126.9, 126.9, 136.1,139.1,139.1,147.5,158.9 (Ar-13C), 163.5-163.8 (-CONH), 67.6($C_2$), 64.3 ($C_3$H);5b,14.8($CH_3$),5e,5f: 55.5-56.7 ($-OCH_3$). The C, H, N analysis data of all compounds are presented in Table -2.

The examination of data reveals that the elemental contents are consistence with the predicted structure shown in Figure-1.The IR data also direct for assignment of the predicted structure. The final structure of all compounds is confirmed by LC-MS data of selected samples. The LC-MS of samples **5b** and **5e** give the molecular ion peak (m/z) at 421 and 434 respectively. These values are corresponds to their molecular weight.

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## BIOLOGICAL SCREENING

### Antibacterial Activities

Antibacterial activities of all the compounds were studied against gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*) and gram-negative bacteria (*E.coli, and klebsiella promioe*) at a concentration of 50µg/ml by agar cup plate method. Methanol system was used as control in this method. Under similar condition using tetracycline as a standard for comparison carried out control experiment. The area of inhibition of zone measured in mm. Compound **5e** and **5f** were found more active against the above microbes. Other compounds found to be less or moderate active than tetracycline (Table -3).

**Table: 3 Antibacterial Activity of Compounds (5a-f)**

| Compounds | Gram +Ve | | Gram -Ve | |
|---|---|---|---|---|
| | ***Bacillus subtilis*** | ***E.coli*** | ***Klebsiella promioe*** | ***Staphylococcs aureus*** |
| **5a** | 51 | 66 | 57 | 55 |
| **5b** | 56 | 67 | 52 | 61 |
| **5c** | 62 | 56 | 58 | 62 |
| **5d** | 60 | 61 | 59 | 56 |
| **5e** | 73 | 64 | 62 | 71 |
| **5f** | 70 | 70 | 61 | 67 |
| **Tetracycline** | 79 | 78 | 86 | 67 |

### Antifungal Activities

The fungicidal activity of all the compounds was studied at 1000 ppm concentration in vitro. Plant pathogenic organisms used were *Rhizopus nigricum*, *Aspergillus niger, Fusarium oxyporium and Botrydepladia thiobromine*. The antifungal activity of all the compounds (**5a-f**) was measured on each of these plant pathogenic strains on a potato dextrose agar (PDA) medium. Such a PDA medium contained potato 200gm, dextrose 20gm, agar 20gm and water one liter. Five days old cultures were employed. The compounds to be tested were suspended (1000ppm) in a PDA medium and autoclaved at $120^{o}$ C for 15 min. at 15atm.pressure. These medium were poured into sterile Petri plates and the organisms were inoculated after cooling the Petri plates. The percentage inhibition for fungi was calculated after five days using the formula given below:

$$\textbf{Percentage of inhibition} = 100(X-Y) / X$$

Where, X = Area of colony in control plate
Y = Area of colony in test plate

The fungicidal activity displayed by various compounds (**5a-f**) is shown in Table-4.

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**Table: 4 Antifungal Activities of Compounds (5a-f)**

| Zone of Inhibition at 1000 ppm (%) | | | | |
|---|---|---|---|---|
| **Compounds** | ***Rhizopus Nigricum*** | ***Aspergillus niger*** | ***Fusarium oxyporium*** | ***Botrydepladia Thiobromine*** |
| **5a** | 55 | 61 | 67 | 64 |
| **5b** | 66 | 57 | 65 | 62 |
| **5c** | 67 | 62 | 68 | 64 |
| **5d** | 69 | 51 | 67 | 65 |
| **5e** | 74 | 69 | 69 | 69 |
| **5f** | 76 | 66 | 71 | 68 |

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