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ChemTech

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.1, No.4, pp 1194-1199, Oct-Dec 2009

SYNTHESIS AND ANTIFUNGAL ACTIVITY OF SOME AZETIDINONES

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ABSTRACT: Ethyl-1H-benzotriazol-1-acetate **2** was prepared by reacting 1H-benzotriazole **1** in alcohol with ethyl bromoacetate in presence of anhydrous potassium carbonate, which was treated with hydrazine hydrate under reflux for 18-24 hours to obtain the intermediate 1H-benzotriazole-1-acetic acid hydrazide **3**. This compound on Schiff's reaction with aromatic aldehydes in presence of solvent mixture yielded 2-(1H-benzotriazol-1-yl)-N'-(substituted phenyl/heteroaryl methylidene) acetohydrazide **4a-4j**. This on reaction with monochloroacetyl chloride and triethylamine in dioxane at low temperature followed by heating at more than 100^{0}C temperature gave N-substituted--2-azetidinones **5a-5j** and were characterized. .The synthesized compounds exhibited moderate to good antifungal activity when tested in vitro against *C.albicans*. Compounds **5g** and **5h** were found to be the most active among all the compounds. To understand their interaction with receptor, these were docked into active site of CYT P-450(PDB-code:1EA1).
KEYWORDS: 1H-Benzotriazole, Azetidinones, Schiff's reaction, Antifungal.

INTRODUCTION

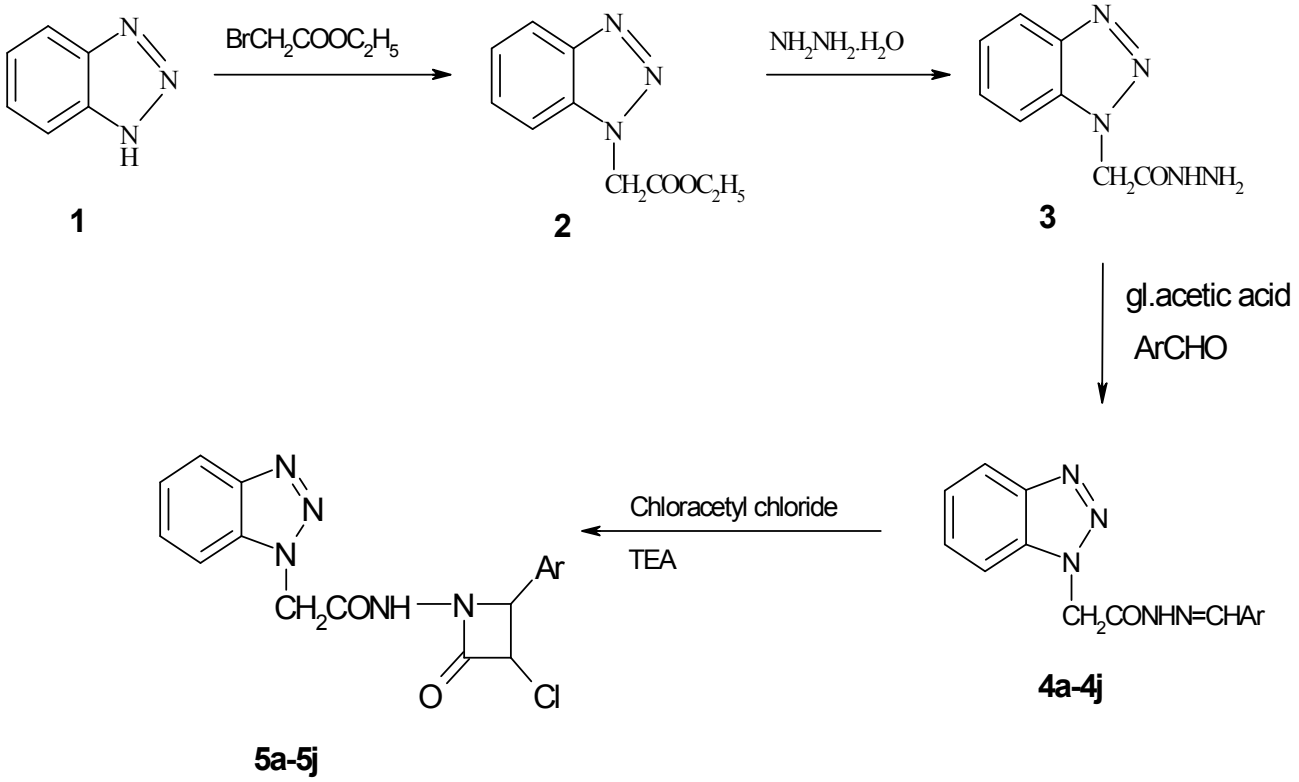
The β-lactam heterocycles are still the most prescribed antibiotics used in medicine[1]. A comparative study of current antibiotics with those from previous decades shows an alarming increase in bacterial resistance to β-lactam antibiotics[2] . Azetidinones, which are part of antibiotics, are known to exhibit interesting biological activities[3]. A large number of 3-chloro monocyclic β-lactams possess powerful antibacterial, antimicrobial, anti-inflammatory, anticonvulsant and antitubercular activities[4]. Also the importance of benzotriazole derivative as chemotherapeutic agents is well established and are associated with potent biological activities as antimicrobial, analgesic ,anticancer activities[5]. With the objective to synthesize and assess the pharmacological profile of new class of compounds it was thought to incorporate 1H- benzotriazole and azetidinone moieties in a single molecule framework. The present work deals with the synthesis of Schiff bases [6,7] starting from 1H-benzotriazole followed by cyclization into azetidinones.The new compounds were then tested for antifungal activity against Candida albicans. Glide was used to observe binding interaction of active compounds into active site of CYTP-450[8] .

BIOLOGICAL EVALUATION

ANTIFUNGAL ACTIVITY

All the compounds were screened for invitro antifungal activity against *C.albicans* using cup-plate agar diffusion method[9] by measuring the zone of inhibition in mm. Fluconazole was used as a standard and was also screened under similar conditions for comparison. A stainless steel borer of 8 mm diameter (pre-sterilized) was used to bore the cavities. Dimethylsulphoxide (DMSO) was used as a solvent for all the compounds and as a control. The testing was performed in Sabouraud's Chloramphenicol Agar medium at concentrations 150, 100, 75 and 50 µg/ml. The plates were incubated at 37 ^{0}C for 24 hrs. The zone of inhibition was observed and measured. The results are presented in Table 2.

SCHEME OF SYNTHESIS



EXPERIMENTAL

GENERAL

All the chemicals and solvents were obtained from commercial source and purified using standard procedure whenever required. Melting points were determined in open capillary tube on VEEGO (VMP-D) melting point apparatus and are uncorrected. IR spectra (KBr pellets) were recorded on a SHIMADZU FTIR 8400S infrared spectrophotometer. The ^{1}H-NMR spectra were determined in DMSO-d_6 at 300 MHz on a BRUKER DP-X 300 NMR spectrophotometer using TMS as an internal standard. The progress of the reaction and the purity of compounds were monitored by TLC using silica gel plates. Microwave assisted reactions were carried out in a "CATALYST SYSTEM" microwave oven.

PREPARATION OF ETHYL-1H-BENZOTRIAZOL-1-ACETATE[10] 2

To a solution of 1H-benzotriazole **1** (0.05 mole) in ethanol (40 ml), ethyl bromoacetate (0.05 mole) and anhydrous potassium carbonate (3 g) were added and the reaction mixture was heated under reflux for 10-14 hours. The solution was filtered and the filtrate was concentrated. The title product was isolated after cooling. Yield 89%; m.p.41^{0}C**,** IR (cm^{-1}): ν_{max} 2985,1747

PREPARATION OF 1H-BENZOTRIAZOLE-1-ACETIC ACID HYDRAZIDE[11] 3

To a solution of ethyl 1H-benzotriazole-1-acetate **2** (0.01 mole) in ethanol (15 ml) hydrazine hydrate (0.02 mole) was added and the reaction mixture was heated under reflux for 18-24 hours. The solution was concentrated and residue obtained thereof was added to ice-cold water and titled product was isolated.

Yield 56%; m.p. 172-173^{0}C,IR(cm^{-1}): ν_{max} 3200**,**1647

GENERAL PROCEDURE FOR PREPARATION OF 2-(1H-BENZOTRIAZOL-1-YL)-N'-(SUBSTITUTED PHENYLMETHYLIDENE) ACETOHYDRAZIDE. (4a-4j)

To a suspension of 1H-benzotriazole-1-acetic acid hydrazide **3** of (0.01 mole) in ethanol and dioxane mixture (2:1), substituted aromatic aldehydes (0.015 mole) and glacial acetic acid (5 ml) were added. The reaction mixture

was heated under reflux for 8-10 hours. After completion of reaction, the reaction mixture was allowed to cool and poured over crushed ice. The precipitated solid thus obtained was filtered, washed with ice-cold water and recrystallized from ethanol.

GENERAL PROCEDURE FOR SYNTHESIS OF 2-(1*H*-BENZOTRIAZOL-1-YL)-*N*-[3-CHLORO-2-(SUBSTITUTED PHENYL)-4-OXOAZETIDIN-1-YL] ACETAMIDE (5a-5j)

A solution of 2-(1H-benzotriazol-1-yl)-N'-(substituted phenylmethylidene) acetohydrazide **4** (0.01 mole) in dioxane (20 ml) was added to a well-stirred mixture of monochloroacetyl chloride (0.01 mol) and triethylamine (0.01 mol) in dioxane at 0-5^{0}C. The mixture was refluxed till completion of reaction. The reaction mixture was then poured into water and titled compounds were isolated. Table 1 summarizes the physical and analytical data of these compounds.

MOLECULAR MODELING

Docking studies were undertaken to gain insight into the binding mode of the most active compounds. The enzyme structure of CYTP-450 was downloaded from PDB. Glide available within Schrödinger software 9.0 was used. Of this series,2-(1H-benzotriazol-1-yl)-N-[3-chloro-2-(2-chlorophenyll)-4-oxoazetidin-1-yl]acetamide.(5g) and 2-(1H-benzotriazol-1-yl)-N-(3-chloro-2-oxo-4-pyridin-3-ylazetidin-1-yl) acetamide (5h) were found to be the most active according to in vitro antifungal testing. During docking studies of compound 5h, it was observed that there is binding interaction between nitrogen atom from azetidinones and heme and also between NH from acetamide and heme .The carbonyl carbon and chloride from azetidinone ring was found in proximity to the amino acids Thr 262 and Ala 256.The 1st nitrogen atom from benztriazolyl ring was found in proximity to Ala 256 and 3rd nitrogen from benztriazolyl ring in proximity of porphyrin ring. The binding interaction of compound 5h and compound 5g was shown in Fig 1 and Fig 2 respectively. The resulting docking scores are given in Table 3.

RESULTS AND DISCUSSION

Ethyl-1H-benzotriazol-1-acetate **2** is prepared by reaction of 1H-benzotriazole **1** in alcohol with ethyl bromoacetate in presence of anhydrous potassium carbonate. The identity of the compound **2** was determined by IR spectrum as sharp strong absorption band at 1745 $^{cm-1}$ due to the ester function. Ethyl 1H-benzotriazole-1-acetate **2** in alcohol when reacted with hydrazine hydrate under reflux for 18-24 hours yields the intermediate 1H-benzotriazole-1-acetic acid hydrazide **3**. The intermediate compound 1H-benzotriazole-1-acetic acid hydrazide **3** is then reacted with aromatic aldehydes in presence of solvent mixture such as alcohol and dioxane at acidic pH to get various Schiff's bases as 2-(1H-benzotriazol-1-yl)-N'-(substituted phenylmethylidene) acetohydrazide **4a-4j.** The IR spectrum of various Schiff's derivatives exhibited absorption band at around 1605 due to –C=N. Compound **4** is mixed with well-stirred mixture of monochloroacetyl chloride and triethylamine in dioxan at low temperature followed by heating at more than 100^{0}C temperature to yield azetidinones. The I.R spectral data of 2-(1*H*-benzotriazol-1-yl)-*N*-[3-chloro-2-(4-chlorophenyl)-4-oxoazetidin-1-yl]acetamide **(5a)** showed sharp bands at 3093(NH *str),* 1676(C=O),1593(NH bending),813(C-Cl). The 1H NMR spectral data for 5a was 2.48 (CH-Cl), 5.59(CH-Ar), 8.7(CO-NH), 6.05(N-CH_2), 7.4-7.52 (phenyl, CH), 7.7-7.84 (benzotriazolyl CH). Subsequent purification yielded final compounds in moderate to higher yields. All the compounds showed good to moderate in vitro antifungal activity when tested against *C. albicans*. The binding interaction of active compounds was studied by docking them into the active site of enzyme cytochromeP-450.The binding scores for various derivatives was given in Table 3.

CONCLUSION

2-(1*H*-benzotriazol-1-yl)-*N*-[3-chloro-2-(substituted aryl/heteroaryl)-4-oxoazetidin-1-yl] acetamides were synthesized and characterized successfully. Derivatives **5h, 5i** and **5j** exhibited maximum activity, which was comparable with standard. Compounds 5h, 5i, 5j are having aryl/heteroaryl ring as substituents which imparts lipophilicity to the molecule. Compounds **5a-5g** and **5i** possesses various substituents on the phenyl ring. Compounds having electron-donating substituents as hydroxy, methyl and methoxy group showed good invitro antifungal activity than the compounds having electron-withdrawing substituents such as nitro group. The docking results also confirmed the interaction of compounds 5g and 5h in the active site of the CYTP-450.Hence it can be concluded that the newly synthesized azetidinones can be novel and potential anti-infective compounds.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. Mandar Kodgule and Dr Sanjay Toshniwal for spectral data and Mr. Sandip Nikam and Ms. Shweta Dhanashire for biological activity.

TABLE 1: PHYSICOCHEMICAL CHARACTERISTICS OF TITLED COMPOUNDS ((5a – 5j)

Compounds	Ar	m.f.(m.w.)	m.p(°C) (%yield)	IR(cm^{-1})	^{1}H NMR
5a	4-Cl-C_6H_4-	$C_{17}N_5O_2H_{13}C_2$ (389)	230°C (95%)	3093(NH*str*),1676 (C=O), 1593(NH bending),813(C-Cl).	2.48 (s,1H,CH-Cl), 5.59(s,1H,CH-Ar), 8.7(s,1H,CO-NH), 6.05(s,2H,N-CH_2) 7.4-7.52(m,4H,phenyl), 7.7-7.84(m, 4H,benzotriazolyl)
5b	4-N$(CH_3)_2$-C_6H_4-	$C_{19}N_6O_2H_{19}Cl$ (396)	221°C (38%)	2941 (NH),1689 (C=O),1608 (CONH), 813 (C-Cl).	8.5 (s, 1H,CONH),2.8-3.07 (m,7H,N-CH_3 and CH Cl), 6.7 (m,2H,N-CH_2),5.9 (s,1H,CH-Ar), 7.2-7.7(m,8H, phenyl , benzotriazolyl
5c	4-OCH_3-C_6H_4-	$C_{18}N_5O_3H_{16}Cl$ (385)	210°C (40%)	2941(NH),781(C-Cl)	3.8 (s,3H,OCH_3),8.6 (s,1H,CONH), 2.48 (s,1H,CH-Cl), 7-7.04(m,4H,phenyl), 7.7-7.8 (m,4H,benzotriazolyl).
5d	2-OH-C_6H_4-	$C_{17}N_5O_3H_{14}Cl$ (371)	220°C (45%)	3083(NH),1691(C=O),1622(CONH),1353(C-O),3388(OH),783(C-Cl).	-
5e	3-NO_2-C_6H_4-	$C_{17}N_6O_4H_{13}Cl$ (400)	250°C (100%)	3087 (NH),1697 (C=O),1525 (CONH),1348 (C-NO2).	8.6 (s,1H,CONH),4.25 (s,1H,CH-Ar),3.02 (s,1H,CH-Cl),7.1-7.6 (m,4H,phenyl),7.7-8.1 (m,4H,benzotriazolyl),6.0 (s,2H,N-CH2)
5f	4-CH_3-C_6H_4-	$C_{18}H_{16}N_5O_2Cl$ (369)	(30%)	2962.46(NH),1679 (C=O),1606 (CONH)	8.6 (s,1H,CONH),3.365(s,3H,CH_3), 2.353(s,1H,CH-Cl),6.052 (m,2H,N-CH_2),5.6 (s,1H,CH-Ar),7.2-7.8 (m,8H, phenyl ,benzotriazolyl)
5g	2-Cl-C_6H_4-	$C_{17}H_{13}Cl_2N_5O_2$ (390)	220°C (90%)	3000 (NH),1701 (C=O),1554 (CONH).	8.73 (s,1H,CONH),5.7(s,1H,CH-Ar),2.56(s,1H,CH-Cl),7.5-7.9 (m,4H,phenyl),8-8.1 (m,4H,benzotriazolyl),6.161 (s,2H,N-CH_2)
5h	C_5H_4N-	$C_{16}H_{13}ClN_6O_2$ (356)	(45%)	2950 (NH),1731 (C=O),1683 (CONH),1558 (C-N).	8.7 (s,1H,CONH),5.65 (s,1H,CH-Ar),3.04 (s,1H,CH-Cl),7.4-7.9 (m,4H,phenyl),7.9-8.5 (m,4H,benzotriazolyl),6.7-6.8 (s,2H,N-CH_2)
5i	4-OH-C_6H_4-	$C_{17}H_{14}N_5O_3Cl$ (371)	(30 %)	3600(OH),2925.81 (NH),1724 (C=O),1600 (CONH).	-
5j	$C_{14}H_9$-	$C_{25}N_5O_2H_{18}Cl$ (455)	(76%)	3087(NH),1697 (C=O),1525 (CONH).	8.6 (s,1H,CONH),4.25 (s,1H,CH-Ar),3.02 (s,1H,CH-Cl),7.1-7.6 (m,4H,phenyl),7.7-8.1 (m,4H,benzotriazolyl),6.0 (s,2H,N-CH_2)

TABLE 2: ANTIFUNGAL ACTIVITY AGAINST *C.albicans*

Compound	150 µg/ml	100 µg/ml	75 µg/ml	50 µg/ml
5a	22	25	24	25
5b	24	20	13	15
5c	23	22	12	13
5d	25	19	20	16
5e	21	14	11	10
5f	20	22	20	15
5g	25	22	18	15
5h	35	30	32	20
5i	30	25	20	22
5j	30	40	40	32
Fluconazole	35	29	32	32

Zone of Inhibition in mm.

TABLE : 3 DOCKING SCORE OF COMPOUNDS WITH CYTP-450

Compound	Docking Score
5a	-6.04
5b	-
5c	-5.95
5d	-9.08
5e	-7.70
5f	-6.49
5g	-9.46
5h	-9.32
5i	-6.97
5j	-
Fluconazole	-8.90

Figure 1: Docking of 2-(1H-benzotriazol-1-yl)-N-(3-chloro-2-oxo-4-pyridin-3-ylazetidin-1-yl)acetamide (5h) in the active site of CYTP-450.

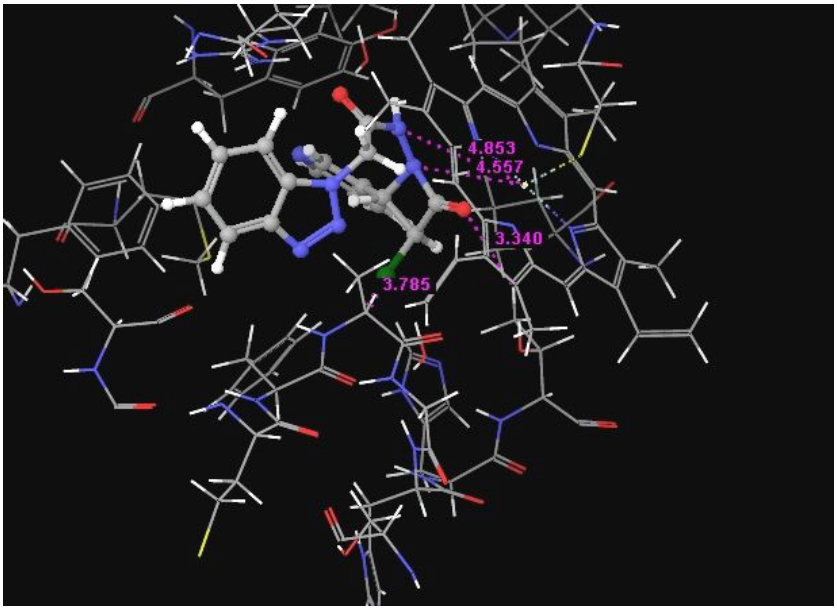
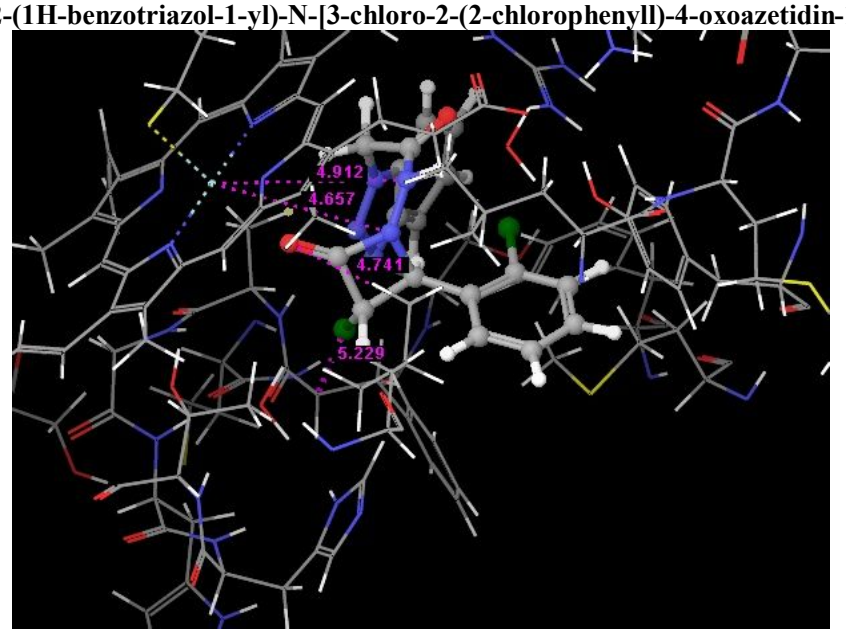


Fig: 2 Docking of 2-(1H-benzotriazol-1-yl)-N-[3-chloro-2-(2-chlorophenyll)-4-oxoazetidin-1-yl]acetamide.(5g)



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