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Research Paper

Synthesis And in-Vitro Anti Bacterial Activity of 2-(2-Benzyl-4-Chlorophenoxy) Acetohydrazide As Triazole Derivatives

Pharmaceutical Science

KEYWORDS : Antibacterial activity; Ethyl bromo acetate; 2-(3-hydroxybenzoyl) benzoic acid

Rohit Bhor

Department of Pharmaceutical Chemistry, PRES's College of Pharmacy Chincholi, Tal-Sinner, Dist-Nasik, Sinner Pin Code-422103, Maharashtra, India.

ABSTRACT

Triazole has been reported to play an important role as antibacterial, antifungal and anti-inflammatory activity. 2-(2-benzyl-4-chlorophenoxy) acetohydrazide derivatives were synthesized and screened for antibacterial activity. The antibacterial activities of carbothioamide derivatives, was tested by disc diffusion method. All the compounds tested against bacteria showed comparable or less antibacterial activities than the reference drug. More specifically, best antibacterial activity & antifungal activity among synthetic analogues was shown by compound AA, AB, AC, CA, CB, and CC, possess very good activity against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa at concentration of 100 mg/ml with Gentamycin was used as standard for comparison of antibacterial activity.

INTRODUCTION

Thiadiazole and Triazoles heterocyclic compounds were reported with wide range of biological activities. Thiadiazole, Carbothioamide and its derivatives are important heterocyclic in organic and biochemistry. Many Triazoles derivatives have shown interesting biological properties such as antibacterial, anti-inflammatory, antioxidant, antitumor, antifungal and immune suppressant activities. It reveals that Triazoles posses broad spectrum activity such as antimicrobial[1-4], anti-inflammatory[5],analgesic[6], antitumorial[7], antihypertensives[8], anticonvulsant and antiviral[9]. Heterocyclic compounds containing Nitrogen gives a variety of biological activities; antimicrobial activity[10]. Nitrogen containing heterocyclic compounds has received considerable attention due to their wide range of pharmacological activity[11].

MATERIALS AND METHODS

Materials:

2-benzyl-4-chlorophenol, Ethyl bromo acetate, Aromatic isothiocyanates, Pyridine, Hydrazine Hydrate, Sodium hydroxide, Ethanol, Con.Hydrochloric acid, Con. Sulphuric acid i.e. H_2SO_4 etc.

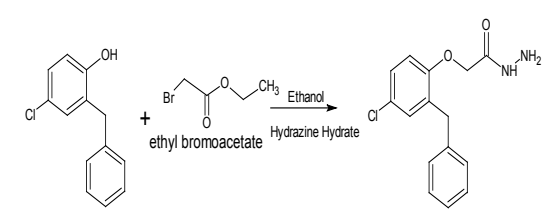
Method:

All Triazole and Carbothioamide derivatives were synthesized by conventional method. Melting points were determined by open tube capillary method. The purity of the compounds was checked on thin layer chromatography (TLC) plates (silica gel G) in chloroform: acetone (8:2) and chloroform: methanol (8:2) solvent systems, the spots were located under iodine vapors and UV light.

General procedure for Synthesis of 2-(2-benzyl-4-chlorophenoxy) acetohydrazide (ADH): [Scheme 1]

A mixture of 2-benzyl-4-chlorophenol (0.5g) and Ethyl bromo acetate (0.5g) react with each other in the presence of Ethanol (15 ml) and hydrazine hydrate and reflux for 3 hrs and then Completion of the reaction were confirmed by TLC. The mixture was cooled by addition of ice. The precipitate formed was washed with water and recrystalized from ethanol.

Scheme 1: Synthesis of 2-(2-benzyl-4-chlorophenoxy) acetohydrazide (ADH) from 2-benzyl-4-chlorophenol (ADH)



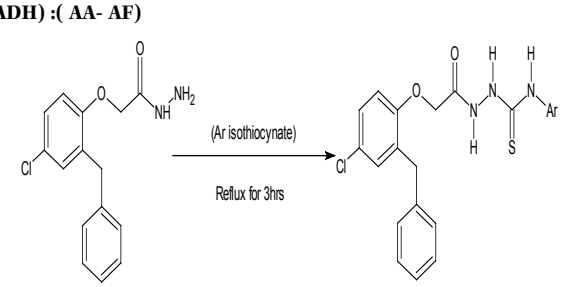
2-benzyl-4-chlorophenol

2-(2-benzyl-4-chlorophenoxy)acetohydrazide

General procedure for synthesis of carbothioamide (AA to AF):[Scheme 2]

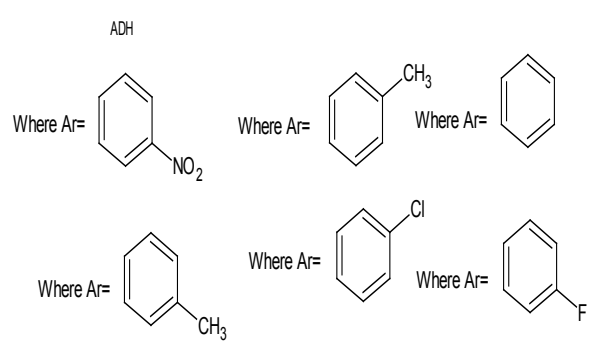
A solution of 2-(2-benzyl-4-chlorophenoxy) acetohydrazide (ADH) reacts with different aromatic isothiocyanates like 3-chlorophenyl isothiocyanates and reflux for 3 hrs and then Completion of the reaction were confirmed by TLC. The mixture was cooled by addition of ice. The precipitate formed was washed with water and recrystalized from ethanol and then it gives different derivatives of carbothioamide like 2-[(2-benzyl-4-chlorophenoxy) acetyl]-N-(3-chlorophenyl)hydrazine carbothioamide

Scheme 2: Synthesis of derivatives of carbothioamide derivatives from 2-(2-benzyl-4-chlorophenoxy) acetohydrazide (ADH) :(AA- AF)



2-(2-benzyl-4-chlorophenoxy)acetohydrazide

Carbothioamide AA to AF



General procedure for synthesis of Triazoles (CA to CF):[Scheme 3]

A solution of different derivatives of carbothioamide likes 2-[(2-benzyl-4-chlorophenoxy) acetyl]-N-(3-chlorophenyl) hydrazine carbothioamide reacts with sodium hydroxide and reflux for 3 hrs and then Completion of the reaction were confirmed by TLC. The mixture was cooled by addition of ice. The precipitate formed was washed with water and recrystalized from ethanol and then it gives different derivatives of Triazoles like 2-[(2-benzyl-4-chlorophenoxy) methyl]-5-(3-chlorophenyl)-1, 3, 4- Triazoles.

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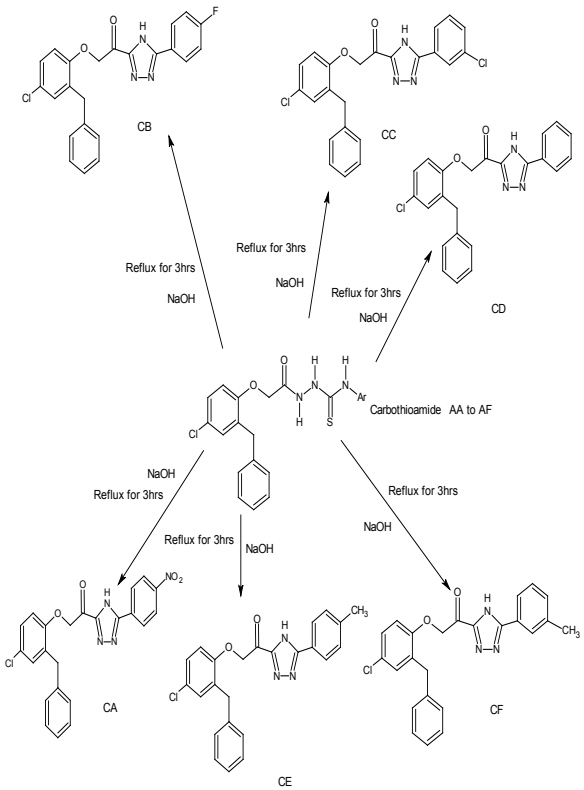

Scheme 3: Scheme for Triazole from carbothioamide derivatives (CA- CF)

Result:

Data Analysis:

2-(2-benzyl-4-chlorophenoxy) acetohydrazide {ADH}:

Colorless solid; $C_{15}H_{19}O_2N_2Cl$; % Yield: 60.15% ; Melting Point: 86-88°C; Rf value: 0.76; FTIR (KBr) ν cm^{-1} : 3063.97 (Ar C-H), 1562.39 (Ar C=C),1631.83 (amide C=O), 1246.06 (Ar C-N), 2946.26 (Aliphatic C-H), 3425.60 (N-H), 775.41 (C-Cl); ^{1}H NMR (500 MHz CDCl3 δ ppm): 7.6-8.2 (m, 3H, aromatic protons), 7.2 (s, 5H, phenyl), 6.4 (s, 1H, NH), 6.8 (s, 2H, NH_2), 4.4 (s, 2H, CH_2), 4.8 (s, 2H, CH_2); FABMS (m/z) 289 (M^+), 290 (M^++1). Mol. Wt.: 290

2-(2-benzyl-4-chlorophenoxy)-1-[5-(4-nitrophenyl)-4*H*-1,2,4-triazol-3-yl]ethanone:(CA)

Colorless solid; $C_{22}H_{16}ON_4Cl$; % Yield: 65.9%; Melting Point: 238°C; Rf value: 0.87; FTIR (KBr) ν cm^{-1} : 3068.85 (Ar C-H), 1591.33 (Ar C=C), 1687.71 (amide C=O), 1234.48 (Ar C-N), 2928.04 (Aliphatic C-H), 3263.86 (N-H), 758.06 (C-Cl); 1HNMR (DMSO, 500MHz): 7.6-8.2 (m, 3H, aromatic protons), 7.2 (s, 5H, phenyl), 4.2 (s, 2H, CH_2, 7.0 (s, 4H, aromatic NO_2), 4.8 (s, 2H, CH_2 Phenyl); FABMS (m/z) 418 (M^+), 419 (M^++1). Mol. Wt.: 419

2-(2-benzyl-4-chlorophenoxy)-1-[5-(4-fluorophenyl)-4*H*-1,2,4-triazol-3-yl]ethanone: (CB)

Colorless solid; $C_{22}H_{16}ON_3ClF$; % Yield: 67.50%; Melting Point: 318 °C; Rf value: 0.94; FTIR (KBr) ν cm^{-1}: 3072.71 (Ar C-H),1537.32 (Ar C=C), 1670.41(amide C=O), 1319.35 (Ar C-N), 2926.11 (Aliphatic C-H), 3267.52 (N-H), 752.28 (C-Cl); 1HNMR (DMSO, 500MHz): 7.5-8.2 (m, 3H, aromatic protons), 7.2 (s, 5H, phenyl), 4.4 (s, 2H, CH_2),7.0 (s, 4H, aromatic F), 4.8 (s, 2H, CH_2); FABMS (m/z) 387 (M^+), 388 (M^++1). Mol. Wt.: 388

2-(2-benzyl-4-chlorophenoxy)-1-[5-(3-chlorophenyl)-4*H*-1,2,4-triazol-3-yl]ethanone: (CC)

Colorless solid; $C_{22}H_{16}ON_3Cl_2$; % Yield: 67.39%; Melting Point: 276 °C; Rf value: 0.79; FTIR (KBr) ν cm^{-1}: 3036.13 (Ar C-H),1583.61 (Ar C=C), 1661.12 (amide C=O), 1257.63 (Ar C-N), 2839.31 (Aliphatic C-H), 3354.31 (N-H), 758.52 (C-Cl); 1HNMR (DMSO, 500MHz): 7.6-8.2 (m, 3H, aromatic protons), 7.2 (s, 5H, phenyl), 4.4 (s, 2H, CH_2),7.0 (s, 4H, aromatic Cl), 4.8 (s, 2H, CH_2); FABMS (m/z) 407 (M^+), 408 (M^++1). Mol. Wt.: 408

2-(2-benzyl-4-chlorophenoxy)-1-(5-phenyl-4*H*-1,2,4-triazol-3-yl)ethanone: (CD)

Colorless solid; $C_{22}H_{17}ON_3Cl$; % Yield: 56.81%; Melting Point: 202°C; Rf value: 0.92; FTIR (KBr) ν cm^{-1} : 2974.77 (Ar C-H), 1599.56 (Ar C=C), 1680.05 (amide C=O), 1392.62 (Ar C-N), 1282.71 (Aliphatic C-N), 2860.88 (Aliphatic C- H), 3309.56 (N-H), 750.18 (C-Cl); 1HNMR (DMSO, 500MHz): 7.6-8.2 (m, 3H, aromatic protons), 7.2 (s, 5H, phenyl), 4.4 (s, 2H, CH_2), 7.0 (s, 4H, aromatic phenyl), 4.8 (s, 2H, CH_2); FABMS (m/z) 372 (M^+), 374 (M^++1). Mol. Wt.: 374

2-(2-benzyl-4-chlorophenoxy)-1-[5-(4-methylphenyl)-4*H*-1,2,4-triazol-3-yl]ethanone: (CE)

Colorless solid; $C_{23}H_{19}ON_3OCl$; % Yield: 71.14%; Melting Point: 298°C; Rf value: 0.94; FTIR (KBr) ν cm^{-1} : 3068.85 (Ar C-H), 1591.33 (Ar C=C), 1687.77 (amide C=O), 1360.78 (Ar C-N), 1234.48 (Aliphatic C-N), 2928.04, 2886.07 (Aliphatic C- H), 3394.83 (N-H), 758.05 (C-Cl); 1HNMR (DMSO, 500MHz): 7.6-8.2 (m, 3H, aromatic protons), 7.2 (s, 5H, phenyl), 4.4 (s, 2H, CH_2), 7.1 (s, 4H, aromatic methyl protons), 1.2 (s, 3H, CH_3), 4.8 (s, 2H, CH_2 Phenyl); FABMS (m/z) 387 (M^+), 388 (M^++1). Mol. Wt.: 388

2-(2-benzyl-4-chlorophenoxy)-1-[5-(3-methylphenyl)-4*H*-1,2,4-triazol-3-yl]ethanone: (CF)

Colorless solid; $C_{23}H_{19}ON_3OCl$; % Yield: 71.14%; Melting Point: 298°C; Rf value: 0.94; FTIR (KBr) ν cm^{-1} : 3038.85 (Ar C-H), 1581.33 (Ar C=C), 1677.77 (amide C=O), 1350.78 (Ar C-N), 1234.48 (Aliphatic C-N), 2828.04, 2886.07 (Aliphatic C- H), 3394.83 (N-H), 738.05 (C-Cl); 1HNMR (DMSO, 500MHz): 7.6-8.2 (m, 3H, aromatic protons), 7.2 (s, 5H, phenyl), 4.4 (s, 2H, CH_2), 7.1 (s, 4H, aromatic methyl protons), 1.2 (s, 3H, CH_3), 4.8 (s, 2H, CH_2 Phenyl); FABMS (m/z) 387 (M^+), 388 (M^++1) Mol. Wt.: 388

PHARMACOLOGICAL STUDIES

Antibacterial Activity

The compounds ADH, CA to CF were evaluated for their *in vitro* antibacterial activity against various microorganisms gram positive *Staphylococcus aureus*, gram negative *Escherichia coli and Pseudomonas aeruginosa by* in vitro method like disc diffusion method was performed using Mueller Hinton Agar (M173) medium. Each compound was tested at concentration 100 µg/mL in DMSO. The zone of inhibition was measured after 24 h incubation at 37°C. Standard: Gentamycin (100 µg/mL of DMSO) (Table-1)

Table 1- In vitro Antibacterial activity screening result of synthesized compound measuring the zone of inhibition in millimeter

Compound No.	Diameter of zone of inhibition (mm)		
	Escherichia coli	Staphylococcus aureus	Pseudomonas aeruginosa
	ATCC 25922	ATCC 25923	ATCC 27853
ADH	15	14	14
CA	19	26	18
CB	19	28	20
CC	18	25	19
CD	15	13	10
CE	14	13	12
CF	15	10	13
Gentamycin	20	36	28

DISCUSSION

IR spectra were obtained on a Perkin Elmer Spectrum1 FT-IR instrument (KBr pellets). Perkin Elmer Spectrum1 FT-IR instrument consists of globar and mercury vapor lamp as sources. ^{1}H-NMR spectra were recorded by a Bruker AVANCE III 500 MHz (AV 500) spectrometer using TMS as internal standard in DMSO-d_6/$CDCl_3$ and mass spectra was obtained on JEOL GCMATE II GC-MS are presented as m/z. The synthetic route for the title compounds is shown in Scheme 1, 2 and 3.Our synthetic strategy for Synthesis of 2-(2-benzyl-4-chlorophenoxy) acetohydrazide {ADH} derivatives is illustrated in general scheme, scheme (1),(2) and scheme (3). Moreover, the compounds having CA, CB, and CC the side chain containing aromatic heterocyclic ring which is attached by Cl, F halogen and NO_2 group showed higher activity than aliphatic group which is attached by CH_3 and phenyl derivatives. It is interesting to note that a minor change in the molecular structure of investigated compounds may have a pronounced effect on antimicrobial screening

CONCLUSIONS

In summary we have synthesized a novel series of 2-(2-benzyl-4-chlorophenoxy) acetohydrazide as Triazole derivatives and evaluated by IR, MS and NMR. The antibacterial activity of the synthesized compounds may be due to the presence side chain containing aromatic heterocyclic ring which is attached by Cl, F halogen and NO_2 group showed higher activity than aliphatic group.

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