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Synthesis and antibacterial activities of 5-subsituted-4-amino-1,2,4-triazole-3-thiols

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Abstract

In the current study, 5-subsituted-4-amino-1,2,4-triazole-3-thiols were prepared from substituted benzoic acid thorough multi step reaction sequence by the cyclization of potassium 3-aroyl-odithiocarbazates obtained from hydrazides, in turn prepared form esters. Structure of synthesized compounds was elucidated by using spectroscopic techniques i-e., UV-Visible, FT-IR, ^{1}H and ^{13}C NMR and mass spectrometry. Results obtained show that 4-amino-5-o-tolyl-4H-1,2,4-triazole-3-thiol, 4-amino-5-m-tolyl-4H-1,2,4-triazole-3-thiol and 4-amino-5-p-tolyl-4H-1,2,4-triazole-3-thiol showed antibacter-ial activity.

Keywords: Antibacterial; triazole; antimicrobial

Introduction

Heterocyclic compounds are very important role in our lives: by their utility in the form of medicinal compounds and in the form of modern materials (Pazharskii et al, 1997). Due to excessive use of hetrocyclic compounds as anti-microbial agent, drug resistance produced in microorganism, which can open a new field for the researchers to prepare the mimic of already existing compounds. 1,2,3-triazoles or 1,2,4-triazoles act as a active pharmaceutical agent used as antitumor (Al Soud et al, 2004), muscle relaxtant, anticonvulsant (Küçükgüzel et al, 2004), antifungal (Al Omran et al, 2002), antibacterial (Rehman et al, 2002), molluscicidal (Radwan et al, 2001), antituberculosis (Foroumadi et al, 2003), diuretic (Lewwnstein et al, 1954), anticancer (Hasejawa et al, 1986), insecticidial (Gupta et al, 1979), hypoglycemic (Holla et al, 1987), antiviral (Kumar et al, 2008), anti-inflammatory, antimic-

robial, antidepressants and antiulceration (Mishra et al, 1991). Biological activity of these compounds was enhanced by using complexes of already available drugs (reference required). Present study was focused to prepare novel substituted compounds of 5-subsituted-4 amino-1,2,4-triazole-3-thiols to overcome the problems of resistance produced in microorganisms.

Materials and Method

All the solvents used were of AR grade. Melting points were determined on Gallenkamp digital point apparatus and are uncorrected. UV spectra were obtained by using Perkin Elmer Lambda 20 Norwalk CT06859 USA. IR spectra were recorded as KBr discs on Bio Rad Elmer 16 FPC FT-IR. H and C spectra were recorded on Jeol JMM-LA 500 FT NMR using $Si(CH_3)_4$ as internal reference.

Synthesis of Esters

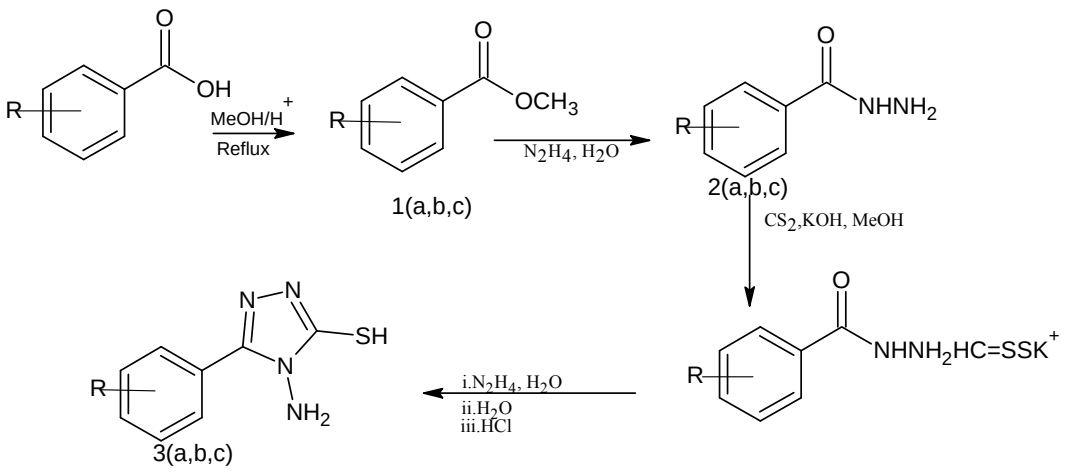
Initially, 0.2 moles substituted benzoic acid was dissolved in 50ml of ethanol in a round bottom flask equipped with a reflux condenser and calcium chloride drying tube. 0.002 moles of concentrated sulphuric acid was added and refluxed for 8hrs. After completion of reaction excessive alcohol was removed and poured in the cold water. The oil layer was separated and washed with dilute sodium carbonate solution to remove un-reacted acid. This washed layer was dried over anhydrous sodium sulphate. Excessive ether was removed by rotary evapora-tor.

Synthesis of Hydrazides

Esters (0.02 moles) as mentioned, were dissolved in 100ml rounds bottom flask fitted with reflux condenser and calcium chloride drying tube. 0.04 moles of hydrated hydrazine was added slowly. After completion of reaction (Scheme 1), mixture was concentrated under reduced pressure. Resulting crude solid was filtered and washed with water and recrystallized from aqueous ethanol.

Synthesis of 5-Subtituted-4-amino-1,2,4-Triazole-3-thiols

A solution of potassium hydroxide (0.04), methanol (100 ml) and aroylhydrazide (0.04 moles) was treated with carbon disulfide (0.04 moles). This mixture was diluted with 150 ml of methanol and stirred for 6 hours at room temperature. Dry ether (200 ml) was added and the precipitated solid was filtered, washed with ether, and vacuum dried at 78°C in drying pestle. The potassium salts prepared as described were obtained in nearly quantitative yield used in the next step without further purification. A suspension of the potassium hydrazine (0.02 moles), hydrazine hydrate (0.04 moles) and 2.0 ml of water was refluxed with stirring for 0.5-1.5 hour. The color of the reaction mixture changed to green, hydrogen sulfide gas was evolved and a homogenous solution was formed. The evolution of hydrogen sulphide gas was evolved and a homogenous mixture was formed. The evolution of hydrogen sulphide gas was tested by its characteristic odor and lead acetate test. After the completion of chemical reaction by the cease of H_2S gas diluted the reaction mixture with 100ml of cold



Scheme 1. Synthesis of 5-subsituted-4-amino-1,2,4-triazole-3-thiols

water and acidified with conc. HCl, a white precipitate formed. Product formed was filtered, washed with cold water and recrystallized from aq. ethanol. (Scheme-1)

Results and Discussion

Spectral Data

1a (2Me): IR (KBr) cm^{-1}: 3060 (Ar-H), 2934 (C-H of CH_3), 1724 (C=O), 1561 (C=C), 2934 (C-R). **1b (3Me)**: IR (KBr) cm^{-1}: 3071 (Ar-H), 2943 (C-H of CH_3), 1729 (C=O), 1577 (C=C), 2943 (C-R). **1c (4Me)**: IR (KBr) cm^{-1}: 3073 (Ar-H), 2935 (C-H of CH_3), 1723 (C=O), 1583 (C=C), 2935 (C-R). **2a**: IR (KBr) cm^{-1}: 3269 (NH_2), 3187(N-H), 1610 (C=O), 1571 (C=C), 2927 (C-R), Rf: 0.71, Yield: 64%. **2b**: IR (KBr) cm^{-1}: 3264 (NH_2), 3182(N-H), 1618 (C=O), 1583 (C=C), 2941 (C-R), Rf: 0.74, Yield: 67%. **2c:** IR (KBr) cm^{-1}: 3272 (NH_2), 3171(N-H), 1635 (C=O), 1563 (C=C), 2936 (C-R), Rf: 0.72, Yield: 76%.

3a: UV λ_{max} nm: 254, IR (KBr) cm^{-1}: 3285 (NH_2), 2564 (N-H/ S-H Str), 1539 (C=N), 1592 (C=C), 2943(C-CH_3), ^{1}H NMR δ ppm: 13.6 (S-H, bs), 7.58-7.51((Ha, Hc, m), 7.45-7.41 (Hb, Hd, m), 5.38 (2H-NH_2,s), 2.48 (1H-CH_3, s), ^{13}C NMR δ ppm: 177.6 (C-1), 161.8 (C-2), 128.7 (C-3), 151.5 (C-4), 126.4 (C-5), 130.8 (C-6), 132.0 (C-7), 138.4 (C-8), 22.0 (C-9), m/e (100%): 206.0, 4.50% (191.0), 18.0% (144.0), 13.0 % (91.0), 13.0 % (89.0), 5.0 % (77.0), Rf: 0.38, Yield: 70% .

3b: UV λ_{max} nm: 253, IR (KBr) cm^{-1}: 3306 (NH_2), 3104(N-H/ S-H Str), 1533(C=N), 1579(C=C), 1325 (C=s), 2954(C-CH_3), ^{1}H NMR δ ppm: 8.23 (1H-Hb, ddd), 8.1 (1H-Ha, d), 7.59-7.52 (2H-Hc, Hd, m), 5.49 (3H-NH_2,bs), 2.61 (4H-CH_3, s), ^{13}C NMR δ ppm: 178.0 (C-1), 167.3 (C-2), 161.8 (C-3), 137.2 (C-4), 126.3 (C-5), 136.5 (C-6), 126.3 (C-7), 137.2(C-8), 21.5 (C-9), m/e (100%):206.0, 7.0% (191.0), 27.0% (144.0), 15.0 % (91.0), 13.0 % (89.0), 4.0 % (77.0), Rf: 0.34, Yield: 69%.

3c: UV λ_{max} nm: 253, IR (KBr) cm^{-1}: 3246 (NH_2), 3146 (N-H/ S-H Str), 1531 (C=N), 1586 (C=C), 1314 (C=s), 2857(C-CH_3), ^1H NMR δ ppm: 8.1 (2H-Ha,a', d), 7.49 (2H-Hb,b', d), 5.6 (2H-NH_2,bs), 2.89 (3H-CH_3, s), ^{13}C NMR δ ppm: 177.4 (C-1), 165.2 (C-2), 149.3 (C-3), 137.2 (C-4), 126.3 (C-5), 136.5 (C-6), 136.3 (C-7), 137.2(C-8), 20.8 (C-9), m/e (100%):206.0, 7.0% (191.0), 27.0% (144.0), 15.0 % (91.0), 13.0 % (89.0), 4.0 % (77.0), Rf: 0.36, Yield: 71%. (Fig-2, 3,4)

Formation of methyl esters were characterized by carbonyl absorption in the region from 736 to 723 cm^{-1}. Esterification was further supported by the disappearance of typical broad OH absorption in the region from 3400 to 2400 cm^{-1} a distinct feature of carboxylic acid.

Conversion of methyl ester in to the hydrazides were confirmed by the appearance of absorption band for primary NH_2 in the region of 3268-3264 cm^{-1} while absorption band for secondary NH was observed at 3212-3171 cm^{-1}. Strong absorption in the region 1654-1610 cm^{-1} was assigned to the carbonyl group of amide linkage.

UV spectrum of ethanolic solution os triazole 3a exhibited λ_{max} at 254nm while triazoles 3b and 3c shows two absorption maxima at 253 and 285nm. This data indicated that triazole 3a existed in the thiol form only and triazoles 3b and 3c existed in thione form in ethanolic solution as the absorption at 285-288nm is due to the chromophoric C-S group.

FTIR of triazoles shows characteristics absorption for NH_2 group with a shoulder appeared at 3306-3246 cm^{-1}. In compounds 3b and 3c an absorption band for N-H stretching vibrations was observed at 3104 cm^{-1} and 3146 cm^{-1} respectively indicating there existence in the thione form while an absorption band for SH absorption was observed at 2564 cm^{-1} for 3a which confirmed its existence in thiol form. This was affirmation of the evidence provided by UV spectroscopy. The disappearance of sharp CO absorption band of hydrazides and appearance of comparatively weak band in the region of 1539-1531 cm^{-1} for C=N was an indication for the formation of triazole ring. Further the appearance of C=S absorption band (3b, 3c) in the region of 1325-1314 cm^{-1} also indicated that cyclization of hydrazides have been taken place. The C-CH_3 absorption band appears in the region of 2927-2945 cm^{-1}.

The proton NMR spectrum of 3a shows two multiplets for four protons in the aromatic region. A muliplet at 7.58-7.51 ppm integrating for two protons was assigned to proton Ha and Hc, while the second mutiplet at 7.45-7.41 ppm was due to two protons Hb and Hd. The singlet for two NH_2 protones was observed at 5.38 ppm. A broad dwon field singlet at 13.64 ppm was assigned to SH protons. The appearance of this signal is in accordance with the observation that the compound existed predominantly in the thiol form. The singlet peak for the CH_3 protons at 2.48 ppm down field was also observed.

The ^1H NMR spectrum of 3b shows four signals for four protons three in the aromatic region while one for the CH_3 protons. A doublet of doublet at 8.1 ppm integrating for one proton was assigned Ha. The coupling constants were in close agreement with meta coupling constants when it couples with Hb and Hc. Another signal at 8.23 ppm with multiplicity, dou-blet of doublet integrating for one proton wa assigned to Hb. A multiplet observed at 7.59-7.52 ppm was assigned to Hc and Hd. A slightly broad band signalat 5.49ppm was assi-

Table-1. Antibacterial Activity of Newly synthesized Compound (Percentage of inhibition).

Compounds	*Staphylococcus aureus*	*Bacillus subtilis*	*Eschercichia coli*	*Pseudomonas aeroginosa*
3a	11	15	9	-
3b	12	18	8	-
3c	10	17	9	-
Amoxicilin	25	34	13	10

gned to NH_2 protons while the singlet peak for CH_3 protons was observed at 2.61 ppm down field. The down field signals for SH proton was not observed confirming the existence in thi-one form.

^{1}H NMR spectrum of 3c shows two doublets in the aromatic region which were in accordance with the expected pattern. A two proton doublet at 8.1 ppm with coupling constant of 8.6 Hz was assigned to Ha and Ha', the second two proton doublet at 7.49ppm with coupling constant of 8.6Hz was assigned to Hb and Hb'. A two proton broad singlet at 5.6ppm was assigned to two NH_2 protons. A three protons singlet peak at 2.89ppm was assi-gned to CH_3 protons.

Proton decoupled ^{13}C NMR spectra of the ligands were recorded in the deutreated solvent chloroform. The ^{13}C NMR shows clearly the respective peaks for the aromatic carbons in the range of 128-149 ppm down field. While the carbon 1 and 2 which were most de hielded in the range of 161-178 ppm down field.

Mass spectral data shows that peak at m/z 206 represents the molecular ion peak and laos the base peak in all the three cases. An important fragment observed at m/z 191 was due to the loss of methyl group common in all the three compounds. The fragment at m/z 77 observed was due to the phenyl ion. While the fragment at m/z 131 and azirine fragment at m/z 89 was formed due to the N-N and N-C bonds homolytic cleavage which confirmed the structures of triazoles.

Antibacterial Activity

All newly synthesized compounds were screened for their antibacterial activity (table 1). Anti-bacterial activity was performed by percentage of inhibition method. Two Gram-positive and two Gram-negative bacteria were taken to evaluate activity of synthesized compounds by using agar diffusion assay (Chaudhry et al, 2012). All activities were done triplicate and mean value was mentioned in table. Results showed that currently prepared thiazoles were excellent active against the Gram-positive bacteria. Out of its activity against the Gram-positive bacteria its activity was best for the *Bacillus subtilis*. Activity of all newly synthesized compounds has close agreement with each other but these compounds were less potent as compared to the reference standard.

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