Synthesis and anti-tubercular evaluation of some pyrrole derivatives

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Abstract

Novel series of pyrrole derivatives were synthesized with an approach to reduce the growing anti-tubercular resistance and to develop more potent and less side effects having anti-tubercular, anti-inflammatory and anticancer activity. An efficient synthesis of different novel 2-amino-4,5-diphenyl-1-substituted-1H-pyrrole-3-carbonitriles derivatives by the Paal-Knorr Condensation of benzoin with primary aromatic amines in refluxing ethanol resulted in the formation of α-amino ketone intermediates, which were condensed without isolation, with malononitrile to yield the various 2-amino-4,5-diphenylpyrrole-3-carbonitriles (1a-d). Pyrroles 1a-d reacted with different reagents such as acetic anhydride, sodium azide, hydroxyl amine hydrochloride to yield compound (1a1-1d1). The synthesized compounds were confirmed through spectral characterization using IR, 1H NMR and Mass. The Pyrrole derivatives examined for their in vitro anti-tubercular testing using Nitrate Reductase Assay. Activity of the synthesized compounds was carried out against H37RV bacteria. Result indicated that these compounds showed promising anti-tubercular activity in comparison to rifampycin (the standard anti-tubercular drug).

Keywords: Paal knorr condensation, Pyrrole derivatives, anti-tubercular, Nitrate Reductase Assay.

1. Introduction

Pyrrole and its derivatives are important heterocycles in organic and bio-chemistry and have been found in many pyrrole-containing natural products such as heam, chlorophyll, vitamin B12 and bile pigments. The pyrrole derivatives are widespread in numerous natural products, and many of them display diverse biological activities. Besides, Pyrrole is one common structural unit in many organic materials. In the preparation of Pyrrole derivatives, however, many disadvantages including harsh reaction conditions and poor yields limit the application of classical methods, such as Knorr reaction and Paal-Knorr reaction. Although some novel strategies have been developed to synthesize Pyrrole derivatives recently. Pyrrole and the simple alkyl Pyrrole are colorless liquids, with relatively weak odors rather like that of aniline, which also like anilines, darkens by auto oxidation. The pyrrole scaffold is an useful structural pattern for exhibiting chemical functionality in biologically active molecules [1a]. It has established broad application in drug development for the treatment as antibacterial, anti-inflammatory, antiviral, antitumor, and antioxidant agent. The pyrrole ring system is one of the most important substructures for biologically active compounds such as indolizidine alkaloids, unsaturated β-lactam and bicyclic lactams. These structural units are found in a wide array of natural products, synthetic materials and bioactive molecules such as vitamin b12, heam and cytochromes therefore, preparation of pyrrole has attracted considerable attention of chemists in recent years. There are several methods for the synthesis of Pyrrole in the literature from classical hantzsch procedure, 1, 3-dipolar cyclo addition reaction, aza-wittig reaction, reductive coupling, titanium catalyzed hydro amination of dynes and other multistep operations. The most widely used method is the Paal-Knorr synthesis, which involves the cyclo condensation reaction of 1, 4- dicarboxyl compounds with primary amines to produce substituted Pyrrole.

2. Material and Method

In 2013 all chemicals and solvents were procured from commercial sources, purified and dried using standard procedures from literature whenever the reagents were purchased from Samarth Lab, Loba Research lab. IR spectra were recorded using KBR disc on JASCO FTIR-410. H’NMR spectra were performed in DMSO solution and their chemical shifts are reported in δ unit with respect to TMS as internal standard at IIT Powai, Mumbai. Mass spectra were obtained from Oxygen Healthcare Research P. Ltd, Ahmedabad in the same year.

2.1 Experimental

Fig. 1 Synthesis scheme of pyrrole derivatives

Step 1:

\[
\text{Benzoin} + \text{substituted aniline} \xrightarrow{\text{Ethanol, reflux}} \text{Product (1a-c)}
\]

Step 2:

\[
\text{Product (1a-c)} \xrightarrow{\text{IR, 1H NMR, Mass}} \text{Pyrrole derivatives (1a1-1d1)}
\]

Derivative of 4, 5- diphenyl pyrrole (1a1, 1b1, 1c1, 1d2, 1h2.)

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Table no. 1 list of substituent’s used in synthesis

<table>
<thead>
<tr>
<th>Substituent</th>
<th>R</th>
<th>R’</th>
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<tbody>
<tr>
<td>I</td>
<td>O-NO₂</td>
<td>2-CH₃OH</td>
</tr>
<tr>
<td>II</td>
<td>3-CH₃</td>
<td>NHCOCH₃</td>
</tr>
</tbody>
</table>

R = Substituent present on pyrrole carbon, R’ = Aniline Substituent

2.1.1 Synthesis of 2-amino-4, 5-diphenyl-1-substituted-1H-pyrrole-3-carbonitriles (1a-c)⁵

A mixture of benzoin (2 g, 0.01 mol), the appropriate amine [α-nitro aniline] (0.93 g, 0.01 mol), p-nitro aniline (1g, 0.01 mole) and conc. HCl (6–8 drops) in ethanol (50 mL) was heated under reflux for 5h, 9h, 10hr, 8hr respectively and cooled. Malanoinitrile (1mL 0.01 mol) was added, followed by a catalytic amount (0.5 mL) of pyridine portion wise and left to reflux until a solid formed. The solvent was evaporated under reduced pressure and the residue was recrystallized from methanol to give compounds 1a-c, respectively. Yield: 37%; M.P. 140-144 °C; IR (KBr) (cm⁻¹) 3430, 3330 (NH₂), 2230 (CN), 3010 (Ar- C-Ha), 1700 (C=O); ¹H NMR (DMSO-d₆,300 MHz) (δ ppm) 5.2 (2H, NH2, D₂O exchangeable), 7.0-7.8 (m, 14H, Ar-H).

2.1.2 Synthesis of N-[1-(2-nitrophenyl)]-3-Cyano-4, 5-diphenyl-1H-pyrrol-2-yl-acetamides (1a1)

The appropriate amino pyrrole, 1a (3.35 g, 0.01 mol), in acetic anhydride (40 mL) was refluxed for 5h, cooled, poured onto ice-water, neutralized with ammonia to give compound N-[1-(2-nitrophenyl)] 3-Cyano-4,5-diphenyl-1H-pyrrol-2-yl) acetamides, respectively in the form of precipitates which were filtered off, dried, and recrystallized from methanol. Yield: 65%; M.P.146-148°C; IR (KBr) (cm⁻¹) 3070 (Ar-C-Ha), 3400(NH), 1620 (C=O); ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm) 5.43 (s, 1H, NH), 3.12 (s, 3H, CH₃), 7.3-7.8 (m,14H, Ar-H).

2.1.3 Synthesis of N-[1-(4-nitrophenyl)]-3-Cyano-4, 5-diphenyl-1H-pyrrol-2-yl-acetamides. (1b1)

The appropriate amino pyrrole, 1b (3.20 g, 0.01 mol), in acetic anhydride (40 mL) was refluxed for 5 h, cooled, poured onto ice-water, neutralized with ammonia to give compound N-[1-(4-nitrophenyl)] 3-Cyano-4,5-diphenyl-1H-pyrrol-2-yl) acetamides, respectively in the form of precipitates which were filtered off, dried, and recrystallized from methanol. M.P.164-166 °C; IR (KBr) (cm⁻¹) 3450, 3380 (NH₂), 3040 (Ar- C-Ha), 1710, 1690 (C=O), and disappearance of the CN group; ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm) 3.43 (s, 3H, CH₃), 5.1 (1H, NH), 7.3-7.8 (m, 14H, Ar-H).

2.1.4 Synthesis of N-[1-(3-ethylbenzyl)]-3-Cyano-4, 5-diphenyl-1H-pyrrol-2-yl-acetamides.(1c1)

The appropriate amino pyrrole, 1c (3.49 g, 0.01 mol), in acetic anhydride (40 mL) was refluxed for 5 h, cooled, poured onto ice-water, neutralized with ammonia to give compound N-[1-(3-ethylbenzyl)] 3-Cyano-4,5-diphenyl-1H-pyrrol-2-yl) acetamides, respectively in the form of precipitates which were filtered off, dried, and recrystallized from methanol. M.P.172-176 °C; 3330 (NH₂), 1720, 1690 (C=O), 3055 (Ar- C-Ha), 2918/(Ali-C-Ha) and disappearance of the CN group; ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm) 5.4 (s, 1H, NH), 5.56 (s, 1H, NH), 5.1(s 2H of NH₂), 4.1 (m, 5H, CH₂-CH₃, D₂O exchangeable), 7.3-7.8 (m, 14H, Ar-H).

2.1.5 Synthesis of 3-[2-amino-1-(p-nitrophenyl)]-4,5-diphenyl-1H-pyrrole) amidoximes(1a2)

The appropriate cyanopyrrole1a (3.49 g, 0.01 mol),hydroxyl amine hydrochloride (0.33 g, 0.01 mol) and anhydrous sodium carbonate (5.3 g, 0.05 mol) in absolute ethanol (40 mL) was refluxed for 4h, filtered while hot and the residue was washed with hot ethanol. The collected filtrate was cooled, poured onto ice-water to yield precipitates, which were filtered, dried, and recrystallized from methanol, to give compound. Yield:67%; M.P. 200-202 °C); 3330 (NH),1730,1700 (C=O), 2730 (OH) 3100 (Ar- C-Ha), and disappearance of the CN group. ¹H NMR (DMSO-d₆, 300 MHz) δ (ppm): 4.3 (s, 2H, NH₂), 4.4 (s, 1H, NH), 4.1 (1H, NH, D₂O exchangeable),7.4-7.9 (m, 14H, Ar-H), 4.5 (s, H, OH).

2.1.6 Synthesis of 3-[2-amino-1-(p-nitrophenyl)]-4, 5-diphenyl-1H-pyrrole amidoxime(1b2)

The appropriate cyanopyrrole1b (3.49 g, 0.01 mol), hydroxyl amine hydrochloride (0.33 g, 0.01 mol) and anhydrous sodium carbonate (5.3 g, 0.05 mol) in absolute ethanol (40 mL) was refluxed for 4h, filtered while hot and the residue was washed with hot ethanol. The collected filtrate was cooled, poured onto ice-water to yield precipitates, which were filtered, dried, and recrystallized from methanol, to give compound.3078.8 (Ar-C-H str), 3358.9 NH, NH₂, 3312.2 OH, 2921.5 Ali-C-Hstr. ¹H NMR(DMSO-d₆, 300 MHz) δ (ppm): 4.3 (s, 2H, NH₂), 4.4 (s, 1H, NH), 4.6 (s, 1H, NH), 4.31 (1H, OH, D₂O exchangeable), 7.3-7.89 (m, 14H, Ar-H).

2.2 Anti-tubercular screening² ³

2.2.1 Inoculation and cultivation of mycobacterium tubercule:

Pure culture of standard strain M. tuberculosis H37Rv procured from Krishna Medical College was inoculated by loop in freshly prepared solid media Löwenstein-Jensen in McCartney bottles procured from Hi media. The media was incubated in incubator at 37°C for 2-3 weeks for cultivation until growth of Mycobacterium tuberculosis in the form of colonies was observed. It was identified and confirmed that the colonies it was only of Mycobacterium tuberculosis by performing acid fast staining.

2.2.2 Sterilization

First sterilized all the required equipments, McCartney bottles and glass wares like conical flask, beakers, volumetric flasks, stirrer etc. in hot air oven at temperature 160°C for 1½ hr, and then removed when it was required for use.

2.2.3 Procedure for preparation of lowenstein-jensen medium:

The most widely used solid medium for TB culture. The modification introduced by the IUATLD is recommended and method used for preparation of LJ media. LJ medium containing glycerol favours the growth of M. tuberculosis while replacement of glycerol by sodium pyruvate enhances the growth of M. bovis and M. africanum.

The following ingredients are aseptically pooled in a large, sterile flask and mixed well to prepare Löwenstein–Jensen media.

1) Mineral salt solution 600 ml
2) Malachite green solution 20 ml
3) Homogenized eggs (20–25 eggs, depending on size) 1000 ml

The prepared medium is distributed in 6–8 ml volumes in sterile McCartney bottles and the tops were securely fastened. Insipissated the medium within 15 minutes of distribution to prevent sedimentation of the heavier ingredients present in the medium.

2.2.5 Preparation of different concentration of drugs:

The different concentrations of standard anti-TB drugs and test samples were prepared (similarly for proportion method and nitrate reductase assay method). Only one concentration per drug was used for standard anti-tubercular drug. The concentrations were as follows:

Rifampicin (40μg/ml): Weighed 5 mg of rifampicin and dissolved in 5 ml of sterile distilled water to make concentration of 1mg/ml as stock solution. Diluted the stock solution with the media to obtain final concentration of 40 μg/ml² ³
2.2.6 Preparation of stock solution of test sample of synthesized compound:

Prepared the stock solutions of each synthesized compound so as to obtain concentration of stock solution was 1mg/ml. Further from stock solution, the different concentrations i.e. 100 μg/ml, 150 μg/ml and 200 μg/ml of each synthesized compound were prepared in different solvents like water, dimethyl formamide and dimethyl sulphoxide according to their solubility.

2.3 Nitrate Reductase Assay (NRA)

2.3.1 Sterilization

Sterilized all the required equipments, McCartney bottles and glassware in hot air oven at temperature 1600°C for 1½ hours and some material like water, cotton was Sterilized by autoclaving.

2.3.2 Procedure

The critical concentrations used were 40 μg/ml for rifampicin and different concentrations (100, 150, 200 μg/ml) of each test sample of the synthesized compound. The LJ media prepared according to procedure described in proportion method and potassium nitrate (KNO₃) 30 mg/ml was added to the media. However growth was not observed of M. tuberculosis H37RV (in the form of pink colour) in control bottles when the reported concentration of KNO₃ 1 mg/ml was used in the media. Hence method was modified as above and growth was observed of M. tuberculosis H37RV (in the form of pink colour) in control bottles. The preparation of each test sample and its incorporation in the media were same as like proportion method. Each different test samples were incorporated in LJ media in such a way that final concentrations obtained were 100, 150 and 200 μg/ml. For each test sample for one concentration three bottles were prepared and used for inoculation of M. tuberculosis H37RV and all the bottles were coagulated same as described in proportion method.

3. Result and Discussion

After addition of 0.5ml of a mixture of three reactants (25 μl of concentrated HCl, 50 μl of 2% sulphanilamide and 50 μl of 1% naphthyl-ethylenediamine dihydrochloride) the compound 1a1, 1a2, 1b1, 1c1, 1b2, 1d1 did not show development of pink color. The compound 1a1, 1a2, 1b1, 1c1, 1b2, 1d1 was found to possess significant anti tubercular activity as compared with Rifampicin.

Figure 2: Observation of growth of M. tuberculosis H37RV (in the form of pink colour)

4. Conclusion

An efficient synthesis of different novel 2-amino-4,5-diphenyl-1-substituted-1H-pyrrole-3-carbonitriles derivatives by the Paal-Knorr Condensation of benzoic acid with primary aromatic amines in refluxing ethanol resulted in the formation of a-amino-ketone intermediates, which were condensed, without isolation, with methanol to yield the various2-amino-4,5-diphenylpyrrole-3-carbonitriles (1a-e). Pyrroles 1a-e reacted with different reagents such as acetic anhydride, sodium azide, hydroxyl amine hydrochloride to yield compound 1a1-1c1. The yield of product 1a-d in the range 37-83% by conventional method and 81-91% by microwave irradiation. The time taken by conventional method was 10 hrs whereas time by the microwave irradiation method was 16 min. at 210 W. The yield of product 1a1-1c1 in the range 43-63% by conventional method and 70-80% by microwave irradiation. The time taken by conventional method was 4 hr where as time by the Microwave irradiation method was 10 min. at 210 W. The structural characterization of the synthesized compounds was done by the interpretation of IR, 1H NMR. All the compounds showed satisfactory IR, 1H NMR. All the synthesized compounds were screened for anti-tubercular activity and they showed good activity.

References

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