Green approach for synthesis of bioactive Hantzsch 1,4-dihydropyridine derivatives based on thiophene moiety via multicomponent reaction

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A novel green and efficient one-pot multicomponent reaction of dihydropyridine derivatives was reported as having good to excellent yield. In the presence of the catalyst ceric ammonium nitrate (CAN), different 1,3-diones and same starting materials as 5-bromothiophene-2-carboxaldehyde and ammonium acetate were used at room temperature under solvent-free condition for the Hantzsch pyridine synthesis within a short period of time. All compounds were evaluated for their in vitro antibacterial and antifungal activity and, interestingly, we found that 5(b–f) show excellent activity compared with Ampicillin, whereas only the 5e compound shows excellent antifungal activity against Candida albicans compared with griseofulvin. The cytotoxicity of all compounds has been assessed against breast tumour cell lines (BT-549), but no activity was found. The X-ray structure of one such compound, 5a, viewed as a colourless block crystal, corresponded accurately to a primitive monoclinic cell.

1. Introduction

More than a hundred years ago, the reaction to produce 1,4-dihydropyridines (1,4-DHPs) was reported by Arthur Hantzsch [1]. These are important precursors due to their pharmacological and biological activities, such as antihypertensive, anti-anginal
and as calcium channel blockade for cardiovascular disease. Consequently, several clinically important drugs appeared on the market with variable new active functional groups in their main skeleton, such as Nicardipine, Nifedipine, Nimodipine, Felodipine, Isradipine and Amlodipine [2–5]. Recently, various attempts have been taken up to improve the Hantzsch reaction using different alternative processes [6–10]. However, most of these reactions were reported with new trends as one-pot multicomponent reactions (MCRs) in the last 10–15 years; these reactions were carried out with certain disadvantages like: longer reaction time, expensive catalyst, higher temperature and tedious workup procedure. Great diversity in MCRs [11–13], developed using various ionic liquids as various Lowry-Bronsted acids, with several advantages has been well documented [14–16]. The clinical significance of pyran, thiophene derivatives of 3-acetylcoumarine based on 1,4-DHPs was the concern that they were reported to have an equivalent cytotoxic effect as the standard CHS 828 against a breast cancer cell line [17].

The above synthetic applications and their medicinal importance encourage us to develop and strive for the enhancement of the pharmacologically bioactive novel 1,4-DHP compounds using MCR. Moreover, the thrust of our work was to enhance the reactivity with suitable electron-releasing and withdrawing groups in reactants to minimize the by-products, and diminish the time of reaction and use of solvent, to establish our protocol with more economy and with a green concept.

In this study, we illustrate our recent work on the synthesis of some novel thiophene-based 1,4-DHP derivatives from different 1,3-diones, and same starting materials as 5-bromothiophen-2-carboxaldehyde and ammonium acetate under room temperature and solvent-free conditions by using ceric ammonium nitrate (CAN) as the catalyst during a short period of time; good to excellent yield is reported with a simple workup procedure, which is shown in scheme 1.

2. Material and methods

2.1. General

All reagents used in this study were purchased from commercially available sources without further purification unless specified otherwise. The synthesized compounds were characterized as anchored in ESI-MS spectra as determined by the Shimadzu GCMS-Qp 2010 spectrometer. Elemental analyses were performed in a Perkin-Elmer 2400 Series-II elemental analyser and the results were within ±0.4% of the theoretical values, unless otherwise noted. The stereochemistry and structure of all compounds were confirmed by $^1$H-NMR and $^{13}$C-APT spectra as recorded on Bruker Advance III ($^1$H: 400 MHz) and ($^{13}$C: 100 MHz), respectively, using CDCl$_3$ as an internal standard. Splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q) and broad (br). The values of chemical shift ($\delta$) were given in ppm and coupling constants (J) in hertz (Hz). IR spectra were recorded on an ABB MB3000 spectrophotometer. Melting points were determined by an open capillary tube melting point apparatus and were uncorrected. The progress of reaction for compounds (5a–f) was monitored by silica gel 60 F254 (Merck)-coated thin-layer chromatography (TLC) plates. Reported $R_f$ values correspond to elution with a 4:1 (n-hexane:ethyl acetate) mobile phase. Some of the compounds were separated
Salmonella typhi; B. subtilis, Bacillus subtilis; S. aureus, Streptococcus aureus catalysed by CAN (ceric ammonium nitrate). (after 15–20 min).

Then the product was poured out and the mixture became solid. We obtained three possible products: added to a 100 ml round bottom flask. The mixture was stirred well for 2.5 h at room temperature; 5-Bromothiophene-2-carboxaldehyde (1.91 g, 0.01 mol), ammonium acetate (0.77 g, 0.01 mol), ethyl-

the mixture became solid. The progress of the reaction was monitored by TLC. The product was washed 1,3-diones (1–2.5 g, 0.01/0.02 mol) and CAN (0.28 g, 0.5 mmol) were added to a 100 ml round bottom flask.

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2.2. Synthesis of 1,4-dihydropyridines (5a–5c & 5g)

5-Bromothiophene-2-carboxaldehyde (1.91 g, 0.01 mol), ammonium acetate (0.77 g, 0.01 mol), different 1,3-diones (1–2.5 g, 0.01/0.02 mol) and CAN (0.28 g, 0.5 mmol) were added to a 100 ml round bottom flask. The mixture was stirred well for 1–2.5 h at room temperature; then the product was poured out and the mixture became solid. The progress of the reaction was monitored by TLC and in the case of compound 5d, we confirmed the products by comparing spots on the TLC plate. We obtained the 5d product in pure form, by dissolving the crude material in a (1:4) mixture of ethyl acetate:n-hexane and separating it by filtration, with a very low amount of filtrate obtained. The remaining crude material may be a mixture of the products 5a and 5b. In the remaining filtrate, we obtained hazy crystals of product 5d (after 15–20 min).

2.3. Synthesis of 1,4-dihydropyridines (5d)

5-Bromothiophene-2-carboxaldehyde (1.91 g, 0.01 mol), ammonium acetate (0.77 g, 0.01 mol), ethyl-acetoacetate (1.3 ml, 0.01 mol), methylacetoacetate (1.1 ml, 0.01 mol) and CAN (0.28 g, 0.5 mmol) were added to a 100 ml round bottom flask. The mixture was stirred well for 2.5 h at room temperature; then the product was poured out and the mixture became solid. We obtained three possible products: 5a, 5b and 5d. Progress of the reactions was monitored by TLC and in the case of compound 5d, we confirmed the products by comparing spots on the TLC plate. We obtained the 5d product in pure form, by dissolving the crude material in a (1:4) mixture of ethyl acetate:n-hexane and separating it by filtration, with a very low amount of filtrate obtained. The remaining crude material may be a mixture of the products 5a and 5b. In the remaining filtrate, we obtained hazy crystals of product 5d (after 15–20 min).

| Table 1. Solvent-free one-pot multicomponent reaction of 5-bromothiophene-2-carboxaldehyde (5BT-2C) and different 1,3-diones catalysed by CAN (ceric ammonium nitrate). |
|---|---|---|---|---|---|
| entry | 5BT-2C | R1 | R2 | time (h) | melting point (°C) | products | R1 value | yield (%) |
| 1 | 5BT-2C | OCH3 | OCH3 | 1.15 | 180–185 | 5a | 0.56 | 77 |
| 2 | 5BT-2C | OCH3 | OCH3 | 1.15 | 140–145 | 5b | 0.59 | 73 |
| 3 | 5BT-2C | OCH3 | OCH3 | 1.15 | 128–142 | 5c | 0.19 | 75 |
| 4 | 5BT-2C | OCH3 | OCH3 | 2.30 | 118–123 | 5d | 0.44 | 51 |
| 5 | 5BT-2C | CH3 | CH3 | 3.00 | 127–132 | 5b + 5c + 5e | 0.40 | 73(21 + 23 + 29°) |
| 6 | 5BT-2C | CH3 | CH3 | 3.00 | 122–125 | 5a + 5c + 5f | 0.46 | 75(22 + 20 + 33°) |
| 7 | 5BT-2C | dimedone | dimedone | 2.30 | 210–213 | 5g | 0.26 | 35 |

*Indicates the % yield of pure compounds 5e and 5f.

| Table 2. Antibacterial activity in microgram per millilitre of the synthesized compounds (5a–5f). E. coli, Escherichia coli; S. typhi, Salmonella typhi; B. subtilis, Bacillus subtilis; S. aureus, Streptococcus aureus; A*, Ampicillin; MTCC, Microbial Type Culture collection. |
|---|---|---|---|---|---|
| compounds | E. coli MTCC443 | S. typhi MTCC98 | S. aureus MTCC96 | B. subtilis MTCC441 |
| 5a | 100 | 250 | 250 | 250 |
| 5b | 200 | 500 | 200 | 100 |
| 5c | 62.5 | 125 | 250 | 200 |
| 5d | 100 | 200 | 100 | 62.5 |
| 5e | 250 | 250 | 100 | 100 |
| 5f | 200 | 100 | 125 | 62.5 |
| A* | 100 | 100 | 250 | 250 |

*Indicates the % yield of pure compounds 5e and 5f.
2.4. Synthesis of 1,4-dihydropyridines (5e & 5f)

5-Bromothiophene-2-carboxyaldehyde (1.91 g, 0.01 mol), ammonium acetate (0.77 g, 0.01 mol), acetylacetone (1 ml, 0.01 mol), ethyl acetoacetate (1.3 ml, 0.01 mol) and CAN (0.28 g, 0.5 mmol) were added to a 100 ml round bottom flask. The mixture was stirred well for 3 h at room temperature; then the product was poured out and the mixture became solid. We obtained three possible products: 5b, 5c and 5e. The progress of the reaction was monitored by TLC and in case of compound 5e, we confirmed the products by comparing spots on the TLC plate. The Rf values for 5b, 5c and 5e are 0.56, 0.20 and 0.46, respectively. Moreover, in 5f we used methyl acetoacetate (1.1 ml, 0.01 mol) instead of ethylacetoacetate and obtained a mixture of the possible products 5a, 5c and 5f. The Rf values for 5a, 5c and 5f are 0.56, 0.20 and 0.46, respectively. The 5e and 5f compounds were separated and purified by column chromatography using an n-hexane: ethyl acetate (4 : 1) mobile phase.

The antimicrobial activity of the synthesized compounds (5a–5f) was evaluated by the broth dilution method. The synthesized compounds were tested for their antibacterial activity against Gram-negative bacteria (E. coli MTCC 443, Salmonella typhi MTCC 98) and Gram-positive bacteria (Bacillus subtilis MTCC 441, Streptococcus pneumonia MTCC 1936). Ampicillin was used as the standard against bacteria. All compounds (5a–5f) were screened for antifungal activity against Candida albicans MTCC 227, Aspergillus niger, MTCC 228 and A. Clavatus MTCC 1323 at a concentration of 500 μg ml$^{-1}$ in DMF. Nutrient agar and potato dextrose agars were used to culture the bacteria and fungi, respectively. The plates were inoculated by the bacteria or fungi and incubated for 24 h at 37°C for bacteria and for 72 h at 27°C for fungi and then the inhibition zones of microbial growth surrounding the filter paper disc (5 mm) were measured in millimetres. Griseofulvin was used as a standard drug for fungi. Test results are given in tables 2 and 3 for antibacterial and antifungal activity, respectively.

3. Results and discussion

We prepared a number of 1,4-DHPs via MCR. According to a literature survey for the development of synthetic procedures [18–21], mainly catalyst can play an important role in the synthesis of 1,4-DHPs...
Therefore, we selected the catalyst CAN to investigate their effect on product yield under solvent-free conditions at room temperature (table 1). Under these optimized reaction conditions, the simplicity and scope of a novel one-pot MCR protocol were explored.

The reaction of 5BT-2C, ammonium acetate as same starting materials and different 1,3-diones was performed in the presence of CAN under solvent-free conditions to obtained desired 1,4-DHP derivatives with good to excellent yield at room temperature. Interestingly, we received the desired symmetrical products 5a, 5b, 5c and 5g by using same 1,3-diones 2 or 3. If we use different 1,3-diones 2,3,
then we get desired asymmetrical products 5d, 5e, 5f including symmetrical products 5a, 5b, 5c and 5f. We obtained a 5d product in pure form, by dissolving the crude material in a (1:4) mixture of ethyl acetate and n-hexane, which was filtered to separate out the undissolved crude, with a very low amount of filtrate obtained. The remaining crude may be a mixture of the products 5a, 5b (monitored by TLC). In the remaining filtrate, we got hazy crystals of product 5d (after 15–20 min).

However, we hypothesized properly pre-functionalized 1,3-dione 2,3 and 5BT-2C. In this series, 5BT-2C contains the electron-withdrawing group –Br, which increases the reactivity of aldehyde to C-C bond formation with 1,3-dione; the presence of a mild to moderate activating group in 1,3-dione increases the nucleophilicity of its methylene group to interact with 5BT-2C, as well as the susceptibility of its carbonyl groups to C-N bond formation by interaction with ammonium acetate. A series of smoothly controlled experiments clearly indicates that the 5BT-2C, ammonium acetate, 1,3-diones and CAN were all necessary substrates for this transformation, as mentioned in scheme 1.

1H-NMR data show signal in a range of 5.1–5.36, which confirms one proton present at the isopolar position on the aliphatic carbon. A singlet was observed at 1–3.6, which confirms the presence of two similar methyl groups in the meta position in the 1,4-dihydropyridine ring, with signals also for methyl
and ethyl groups of esters at the desired positions. The signals of a doublet appears at 6.0–7.08, which confirmed the presence of aromatic protons. 13C-APT spectra show peaks in a range of 13–208, which confirm the presence of methyl groups. Signals at 34–356 confirm the presence of aliphatic –CH group, whereas carbon in the pyridine ring at a double bond gave a signal at 100–1106. Signals at 120–1308 indicate aromatic –CH groups and signals at 165–1708 confirm the presence of carbonyl groups. In the IR data, we obtained a peak near 3340 cm
\(^{-1}\), which is for N–H stretching, and a peak around 3000 cm
\(^{-1}\) for tertiary –C–H stretching, whereas peaks that appear between 1700 and 1600 cm
\(^{-1}\) indicate –C=O stretching. A peak that appears between 1350 and 1300 cm
\(^{-1}\) indicates aromatic olefin stretching. The peak that appears near 1200 cm
\(^{-1}\) provides the proof of –C–H stretching of the methyl group. The peak that appears at 510 cm
\(^{-1}\) indicates –C–X (C–Br) stretching and some peaks observed between 1000 and 700 cm
\(^{-1}\) confirmed the aromatic ortho- and para-di-substituents. In mass spectral analysis, we obtained peaks at the particular mass of the compound, which confirmed the present skeleton in the moiety, and different base peaks at m/z = 332, 318, 265, 230, 145, 57 and 45; these m/z values are the major base peaks in all compounds.

All the synthesized compounds 5(a–f) were screened for Gram-positive bacteria and Gram-negative bacteria and results were compared with Ampicillin as the standard drug. Compound 5c was found highly active, and compounds 5a and 5d showed equipotent activity against E. coli compared with the standard. Compound 5f showed equipotent activity with S. typhi. The interesting finding of our work is to observe excellent antibacterial activity of the 5b, 5c, 5d, 5e and 5f compounds against Staphylococcus aureus and Bacillus subtilis compared with the standard drug, whereas compounds 5a and 5c showed equipotent activity. Similarly, the compounds 5(a–f) were screened for antifungal activity with C. albicans and A. niger as the microorganisms; obtained results were compared with griseofulvin as the standard drug shown in table 3. It was found that compound 5e showed higher activity than the standard. Whereas compounds 5b and 5f had equipotent activity with the standard, and the compounds 5a, 5c and 5d had lower activity.

The cytotoxicity of all synthesized compounds was checked against breast tumour cell lines (BT-549) but no activity was found, which is illustrated in table 4. The compound 5a was confirmed by a single crystal XRD analysis, which is shown in figures 1 and 2. The crystallographic experimental data are described in table 5. The packing arrangement of the molecules viewed down in the a-axis is shown along with obtained crystal structure of compound 5a. From these series, three compounds, 5a, 5c and 5f, were selected for anticancer activity at National Cancer Institute (NCI), USA.

4. Conclusion

In brief, we have confirmed a well-organized solvent-free green procedure for the multicomponent synthesis of dihydropyridine derivatives using the catalyst CAN. The result highlighted the solvent-free procedure to be more efficient than the conventional method. The advantages of the Hantzsch pyridine synthesis are shorter reaction times, simplicity of the reaction, good product yield and easy workup procedures with regard to the build-up to the reaction, which is economical and easy, with CAN being a powerful catalyst for the many organic syntheses. The interesting finding of our work is that we obtained excellent antibacterial activity with compounds such as 5b, 5c, 5d, 5e and 5f, which were found highly active against S. aureus and B. subtilis compared with the standard drug Ampicillin, whereas compounds 5a and 5c showed equipotent activity. As far as antifungal activity is concerned, only compound 5e showed higher activity than the standard griseofulvin drug, and compounds 5b and 5f had equipotent activity. The cytotoxicity of all the compounds has been assessed against breast tumour cell lines (BT-549), but no activity was found.

Ethics. We do not require any ethical approval from a local ethics committee to carry out these studies because we carried out some work with the help of other sources.

Data accessibility. We include all the experimental data including spectral characterizations in the electronic supplementary material. ESI and crystallographic details of 5a compound are available on http://dx.doi.org/10.5061/dryad.66gd7 [26], and other figures and electronic supplementary materials are available on https://doi.org/10.6084/m9.figshare.4990370.

Authors’ contributions. H.M.P. designed the study. M.G.S. and H.M.P. synthesized and characterized the compounds, and wrote the manuscript. D.P.R. carried out the biological activity. All authors gave their final approval for publication.

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