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Original article

One step synthesis of pyrido[1,2-*a*]benzimidazole derivatives of aryloxypyrazole and their antimicrobial evaluation

Hardik H. Jardosh, Chetan B. Sangani, Manish P. Patel *, Ranjan G. Patel

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India

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ABSTRACT

A new series of pyrido[1,2-*a*]benzimidazole derivatives bearing the aryloxypyrazole nucleus have been synthesized by base-catalyzed cyclocondensation reaction through multi-component reaction (MCR) approach. All the synthesized compounds were investigated against a representative panel of pathogenic strains using broth microdilution minimum inhibitory concentration (MIC) method for their *in vitro* antimicrobial activity. Reviewing the data, majority of the compounds were found to be active against employed pathogens. SAR study explores that antimicrobial activity is strongly depends on the nature of the substituents at the ether linked aryl ring attached to the pyrazole unit, together with the substituent present on the C_5 of the benzimidazole unit.

1. Introduction

Over the past few decades, the problems posed by multi-drug resistant microorganisms have reached an alarming level in many countries around the world [1]. This has led to a serious challenge to the medical community; hence, the development of new effective antimicrobial agents is an important goal. In pursuit of this goal, our research efforts are focused on the development of novel structural moieties with promising antimicrobial properties [2–7].

Recently, we have been particularly interested in the synthesis of pyrazole incorporating structures for antimicrobial evaluations [8–10], on the premise that functionalized pyrazole is a chemically useful synthon bearing diverse biological activities like antimicrobial [11,12], anti-inflammatory [13], antitubercular [14], antitumor [15] properties.

In the past few years, pyrido[1,2-*a*]benzimidazole derivatives attracted organic as well as medicinal chemists due to their diverse syntheses and various biological activities including perforin inhibition [16], antimalarial [17], antimicrobial, anti-HIV-1 and anticancer properties [18].

From the aforementioned reports, it seemed that a combination of both therapeutically active moieties aryloxypyrazole and pyrido[1,2-*a*]benzimidazole together in a single molecular framework may enhance the pharmacological activity of the title compounds. On the basis of this hypothesis, we report herein the synthesis and antimicrobial activity of novel pyrido[1,2-*a*]benzimidazole derivatives bearing ether linked-pyrazole moiety.

All the synthesized compounds were characterized using FT-IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectrometry and were subjected to antimicrobial screening *in vitro* against a representative panel of eight human pathogens, of which three Gram-positive bacteria (*Bacillus subtilis*, *Clostridium tetani*, *Streptococcus pneumoniae*), three Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*) and two fungi (*Aspergillus fumigatus*, *Candida albicans*) using broth microdilution MIC (minimum inhibitory concentration) method [19].

2. Experimental

All the reagents were obtained commercially and used after further purification. All melting points were taken in open capillaries in a paraffin bath and are uncorrected. The monitoring of the progress of all reactions and homogeneity of the synthesized compounds was carried out by thin layer chromatography (TLC). TLC was run using TLC aluminum sheets silica gel $60F_{254}$ (Merck). Elemental analysis (% C, H, N) was carried out by Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA) and all compounds are within ±0.4% of theory specified. The IR spectra were recorded in KBr on a Perkin-Elmer Spectrum GX FT-IR spectrophotometer (Perkin-Elmer, USA). ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Bruker Avance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using TMS as an internal standard at 400 MHz and 100 MHz respectively. Chemical

* Corresponding author.
E-mail address: patelmanish1069@yahoo.com (M.P. Patel).

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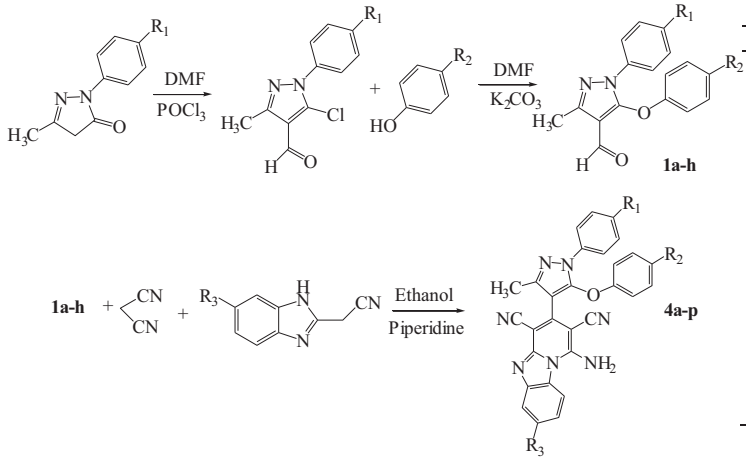

Compd.	R_1	R_2	R_3
4a	H	H	H
4b	H	CH_3	H
4c	H	OCH_3	H
4d	H	Cl	H
4e	CH_3	H	H
4f	CH_3	CH_3	H
4g	CH_3	OCH_3	H
4h	CH_3	Cl	H
4i	H	H	CH_3
4j	H	CH_3	CH_3
4k	H	OCH_3	CH_3
4l	H	Cl	CH_3
4m	CH_3	H	CH_3
4n	CH_3	CH_3	CH_3
4o	CH_3	OCH_3	CH_3
4p	CH_3	Cl	CH_3

Scheme 1. Synthetic pathway for the intermediates **1a**–**h** and compounds **4a**–**p**.

shifts are reported in parts per million (ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan).

General procedure for synthesis of compounds **4a**–**p**: A 100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of aryloxypyrazole-4-carbaldehyde **1a**–**h** (5 mmol), malononitrile **2** (5 mmol), 2-cyanomethylbenzimidazole **3a**,**b** (5 mmol), and a catalytic amount of piperidine (1 mmol) in ethanol (15 mL) (Scheme 1). The mixture was heated under reflux for 3.5 h. and the progress of the reaction was monitored by TLC. After the completion of reaction (as evidenced by TLC), the reaction mixture was cooled to room temperature and stirred magnetically for another 20 min, the solid mass was separated and collected by filtration, washed well with ethanol (15 mL) and purified by leaching in an equal volume ratio of chloroform and methanol (20 mL) to obtain a pure solid sample.

Physical, analytical and spectroscopic characterization data of compounds **4a**–**p** are given in Supplementary Material.

3. Results and discussion

3.1. Chemistry

The synthetic route depicted in Scheme 1 outlines the chemistry part of the present work. The required intermediates, 1-aryl-5-chloro-3-methyl-1*H*-pyrazole-4-carbaldehydes were prepared by Vilsmeier-Haack reaction (chloroformylation) of 1-aryl-3-methyl-1*H*-pyrazol-5(4*H*)-one [20]. 1-Aryl-5-chloro-3-methyl-1*H*-pyrazole-4-carbaldehyde undergoes nucleophilic substitution reaction with respective phenol at refluxing temperature for 4 h in presence of basic catalyst (K_2CO_3) in DMF which resulted in required 3-methyl-5-aryloxy-1-aryl-1*H*-pyrazole-4-carbaldehydes **1a**–**h** according to literature procedure [21,22]. Pyrido[1,2,-*a*]benzimidazole derivatives **4a**–**p** have been synthesized *via* base catalyzed three component cyclocondensation reaction of **1a**–**h**, malononitrile **2** and 2-cyanomethylbenzimidazole **3a**,**b** in ethanol containing a catalytic amount of piperidine in good yield (58%–85%).

The title compounds **4a**–**p** were characterized by ^{1}H NMR, ^{13}C-NMR, FT-IR, mass spectra and elemental analysis. The IR spectrum of title compounds **4a**–**p** revealed the presence of amino, cyano, and ether groups due to the appearance of absorption bands at around 3440–3360 and 3340–3170, 2250–2190, 1230–1180 cm^{-1}, respectively. In ^{1}H NMR spectrum, a singlet around δ 1.90–2.27 appeared for methyl group of pyrazole unit, singlet around δ 2.30–2.40 observed for methyl group of aryl ring attached to pyrazole unit, singlet around δ 2.31–2.38 stands for methyl group of ether linked aryl ring and singlet around δ 2.52–2.58 appeared for methyl group of benzimidazole unit. Singlet around δ 3.38–3.42 stands for methoxy group of aryl ring attached to pyrazole unit. Multiplets in the range of δ 6.60–8.58 appeared for aromatic protons. Moreover, a distinctive broad singlet around δ 8.51–8.75 stands for primary amine of the **4a**–**p** compounds. In the ^{13}C NMR spectral data of the title compounds **4a**–**p**, the signal at around δ 78.40–88.35 is assigned to carbon attached with carbonitrile while signals around δ 102.00–157.00 are attributed to all the aromatic carbon of compounds **4a**–**p**. Also, distinctive signals around δ 13.50–21.85 and δ 55.70–55.75 stands for methyl and methoxy of **4a**–**p** derivatives, respectively. The obtained elemental analysis values are in good agreement with theoretical data. Further, mass spectra of all the title compounds showed molecular ion peak M^+ corresponding to their exact mass.

3.2. Antimicrobial screening

Examination of the antimicrobial data (Table 1) revealed that against Gram-positive bacteria *B. subtilis*, compounds **4e**, **4f**, **4i** and **4m** (MIC = 100 μg/mL) found to be equipotent to ciprofloxacin. Compounds **4b**, **4j** and **4k** (MIC = 200 μg/mL) were found to be more potent compared to ampicillin while compounds **4d**, **4g**, **4o** and **4p** (MIC = 250 μg/mL) were found to have comparable activity to ampicillin. Against the species *C. tetani*, compounds **4l** and **4o** (MIC = 100 μg/mL) were found to possess excellent activity compared to ampicillin and equivalent to ciprofloxacin. Compounds **4d**, **4i**, **4m** and **4n** (MIC = 200 μg/mL) were found to be more active than ampicillin while compounds **4a**, **4c**, **4e**, **4g**, **4j** and **4p** (MIC = 250 μg/mL) were found equally potent to ampicillin. Against the Gram positive bacteria *S. pneumoniae* only compounds **4b**, **4e** and **4m** (MIC = 100 μg/mL) were found to have equal potency compared to ampicillin. Toward Gram-negative strain *E. coli*, compounds **4g** (MIC = 50 μg/mL) and **4p** (MIC = 62.5 μg/mL) were found to have significant activity whereas, compounds **4c**, **4f**, **4i** and **4l** (MIC = 100 μg/mL) were found to have comparable activity, to ampicillin.

Against *S. typhi* compounds **4i** (MIC = 50 μg/mL) and **4f** (MIC = 62.5 μg/mL) were found to have fabulous activity whereas, compounds **4c**, **4h**, **4j** and **4k** (MIC = 100 μg/mL) were found to have comparable activity to ampicillin. Against *V. cholerae* compound **4n** (MIC = 50 μg/mL) was found to be equipotent with chloramphenicol and more potent than ampicillin whereas, compounds **4c**, **4f** and **4p** (MIC = 100 μg/mL) were found to have comparable activity, to ampicillin. Against fungal pathogen *C. albicans*, compounds **4c**, **4g**,

Table 1
In vitro antimicrobial activity MIC (μg/mL) of pyrido[1,2-*a*]benzimidazole derivatives **4a**–**p**.

Compound	Gram-positive bacteria			Gram-negative bacteria			Fungi	
	Bs. MTCC 441	Ct. MTCC 449	Sp. MTCC 1936	Ec. MTCC 443	St. MTCC 98	Vc. MTCC 3906	Af. MTCC 3008	Ca. MTCC 227
4a	500	250	200	250	200	250	1000	500
4b	200	500	100	250	500	500	>1000	500
4c	500	250	500	100	100	100	>1000	250
4d	250	200	250	250	200	250	>1000	1000
4e	100	250	100	500	500	200	>1000	>1000
4f	100	500	250	100	62.5	100	>1000	500
4g	250	250	500	50	250	250	>1000	250
4h	500	500	200	200	100	125	1000	1000
4i	100	200	250	100	50	250	>1000	>1000
4j	200	250	500	250	100	250	>1000	1000
4k	200	125	500	200	100	500	>1000	1000
4l	500	100	500	100	200	250	>1000	500
4m	100	200	100	200	250	250	>1000	250
4n	500	200	200	250	250	50	200	>1000
4o	250	100	250	250	500	200	1000	250
4p	250	250	250	62.5	200	100	>1000	250
Ampicillin	250	250	100	100	100	100	–	–
Ciprofloxacin	50	100	50	25	25	25	–	–
Norfloxacin	100	50	10	10	10	10	–	–
Chloramphenicol	50	50	50	50	50	50	–	–
Griseofulvin	–	–	–	–	–	–	100	500

Bs.: *Bacillus subtilis*; Ct.: *Clostridium tetani*; Sp.: *Streptococcus pneumoniae*; Ec.: *Escherichia coli*; St.: *Salmonella typhi*; Vc.: *Vibrio cholerae*; Af.: *Aspergillus fumigatus*; Ca.: *Candida albicans*.
MTCC: Microbial type culture collection.
'–' represents 'not tested'.

4m, **4o** and **4p** (MIC = 250 μg/mL) were found to be more potent, where as compounds **4a**, **4b**, **4f** and **4l** (MIC = 500 μg/mL) were found to be equipotent when compared with griseofulvin. None of the compounds were found to be active against the fungal pathogen *A. fumigates*. The remaining compounds showed moderate to good activity to inhibit the growth of bacterial pathogens and were found less effective than the employed standard drugs.

The structure–activity relationship study (SAR) indicates that a change in the substituent might also affect the antibacterial activity of title compounds **4a**–**p**. Compounds having R_2=H/CH_3 and R_3=H appeared to have more potential against Gram-positive bacteria *B. subtilis* and *S. pneumonia* as well as against Gram-negative bacteria *S. typhi* and *V. cholerae*. Also, Compounds having R_2=Cl/OCH_3 and R_3=H/CH_3 were found to be more active against Gram-positive bacteria *C. tetani* and Gram-negative bacteria *E. coli* and fungal pathogen *C. albicans*.

4. Conclusion

The engaged synthetic strategy in the present study allows the assimilation of two promising bioactive nuclei into a single scaffold through an easy way. From SAR study, it can be concluded that the antimicrobial activity of title compounds are strongly dependent on the nature of the substituents at the ether linked aryl ring attached to the pyrazole unit, together with the substituent linked to the C_5 of the benzimidazole unit.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2013.01.021.

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