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An efficient synthesis of 3′-indolyl substituted pyrido[1,2-*a*]benzimidazoles as potential antimicrobial and antioxidant agents

HARSHAD G KATHROTIYA and MANISH P PATEL*

Department of Chemistry, Sardar Patel University, Vallabh Vidyanagar 388120, India
e-mail: patelmanish1069@yahoo.com

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Abstract. A new class of indole-based pyrido[1,2-*a*]benzimidazole derivatives **4a–r** have been synthesized by one-pot cyclocondensation reaction of 2-phenyl-*1H*-indole-3-carboxaldehyde **1a–i**, malononitrile **2** and 2-cyanomethylbenzimidazole **3a–b** in the presence of catalytic amount of NaOH. *In vitro* antimicrobial activity of the synthesized compounds were investigated against a representative panel of pathogenic strains specifically three Gram-positive bacteria (*Streptococcus pneumoniae*, *Clostridium tetani*, *Bacillus subtilis*), three Gram-negative bacteria (*Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli*) and two fungi (*Aspergillus fumigatus*, *Candida albicans*) using broth microdilution MIC (minimum inhibitory concentration) method. *In vitro* antioxidant activity was evaluated by ferric-reducing antioxidant power (FRAP) assay method. Compounds **4c**, **4e**, **4l** and **4q** have been found to be most efficient antimicrobial members while compounds **4h** and **4p** possess better ferric reducing antioxidant power.

Keywords. Indole; pyrido[1,2-*a*]benzimidazoles; multicomponent reaction; antimicrobial activity; FRAP assay.

1. Introduction

The treatment of opportunistic microbial infections has become an important and challenging problem due to the emergence of multiple-drug-resistant organisms.[1,2] Hence, there is an urgent need to develop new classes of agents likely to be unaffected by existing resistance mechanisms. Nowadays free metals and radicals become more harmful which play an important role in the pathogenesis of many diseases, accounting for continuing interest in the identification and development of novel antioxidants that prevent metal and radical-induced damages. Antioxidants are of great interest because of their involvement in important biological industrial processes. In general, compounds with antioxidant activity have been found to posses anticancer, anticardiovascular, antiinflammatory and many other activities.[3]

The synthesis of nitrogen-bridge head pyrido[1,2-*a*]benzimidazole ring system was recognized in 1937,[4] a literature survey revealed that most of its derivatives still have unexplored biological activities. Few reports concerning the antineoplastic activity,[5] central GABA-A receptor modulators for the treatment of anxiety,[6] antimicrobial,[7] antiviral,[8] analgesic and antiinflametory.[9–11] Some of them are used as a chemodosimeter for fluoride ions detection[12] and also display interesting photophysical as well as fluorescent properties.[13]

On the other hand, indole derivatives have been a topic of substantial research interest in contemporary heterocyclic and medicinal chemistry due to their great significance in the view of their (i) occurrence in nature as a prominent sub-structure of a large number of alkaloids[14] and (ii) wide-ranging biological activities which includes antimicrobial,[15] antitubercular,[16] ghrelin receptor,[17] antioxidant,[18] antiviral[19] and antimalarial.[20] In particular, 3-substituted indole derivatives play a key role in the synthesis of biologically active compounds especially with anticancer, antitumour, hypoglycemic, antiinflammatory, analgesic and antipyretic activities.[21] Some of the biologically active 3-substituted indole representatives are shown in figure 1.[22]

The molecular manipulation of promising lead compounds is still an organized and chief approach to widen the vicinity of medicine research. It involves an initiative to merge the separate pharmacophoric groups of analogous activity into one compound, thus making structural changes in the biological activity. An attempt

*For correspondence

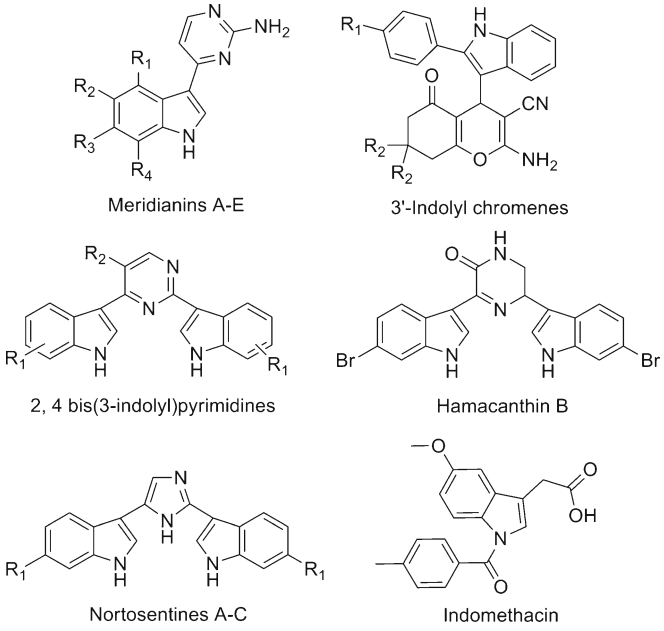


Figure 1. Representative of 3-substituted indoles.

has been made to undertake the synthesis of pyrido[1,2-*a*]benzimidazole derivatives with an assumption that the assimilation of more than one bioactive moieties into a single scaffold may produce novel heterocycles with fascinating antimicrobial activities along with antioxidant activity.

The antimicrobial and antioxidant activity of pyrido[1,2-*a*]benzimidazole derivatives has not been reported to the best of our knowledge. In the radiance of the aforementioned facts and as a prolongation of our investigation on the synthesis of biologically active heterocyclic compounds,[23] we were provoked to synthesize new indole-based pyrido[1,2-*a*]benzimidazoles with various substituents to elucidate their contribution to the antimicrobial and antioxidant activities.

The constitution of all the compounds was characterized using elemental analysis, FT-IR, ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy. All the compounds were screened for *in vitro* antimicrobial activity against eight human pathogens, of which three Gram-positive bacteria (*Streptococcus pneumoniae*, *Bacillus subtilis*, *Clostridium tetani*), three Gram-negative bacteria (*Salmonella typhi*, *Escherichia coli*, *Vibrio cholerae*) and two fungal pathogens (*Candida albicans*, *Aspergillus fumigatus*) using broth microdilution MIC (minimum inhibitory concentration) method according to National Committee for Clinical Laboratory Standards (NCCLS)[24] and ferric reducing antioxidant power (FRAP) was measured by a modified method of Benzie and Strain.[25]

2. Experimental

2.1 *Materials, methods and instruments*

All the reagents were obtained commercially and used with further purification. Solvents were used of analytical grade. All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminum plates coated with silica gel 60 F_{254}, 0.25 mm thickness) (Merck, Darmstadt, Germany) was used for monitoring the progress of all reactions, purity and homogeneity of the synthesized compounds. UV radiation and/or iodine were used as the visualizing agents. 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) and only the characteristic peaks are reported in cm^{-1}. ^{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 solvent peak as internal standard at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in parts per million (ppm). Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan).

2.2 *General procedure for the synthesis of 2-phenyl-1H-indole-3-carboxaldehydes* **(1a–i)**

2-Phenyl-1*H*-indole-3-carboxaldehydes were prepared according to literature procedure[26] by Vilsmeier–Haack reaction of 2-phenyl-1*H*-indoles.

2.3 *General procedure for the synthesis of 2-cyanomethylbenzimidazoles* **(3a–b)**

2-Cyanomethylbenzimidazoles **3a**–**b** were prepared according to literature procedure[27] by reaction of *o*-phenylenediamines and ethylcyanoacetate.

2.4 *General procedure for the synthesis of 1-amino-3-[2-(4-(un)-substitutedphenyl)-1H-indol-3-yl]-7-(un)-substitutedpyrido[1,2-a]benzimidazole-2,4-dicarbonitrile* **(4a–r)**

A 100 mL round bottomed flask, fitted with a reflux condenser, was charged with a mixture of 2-phenyl-1*H*-indole-3-carboxaldehydes **1a**–**i** (1 mmol), malononitrile **2** (1 mmol), 2-cyanomethylbenzimidazoles **3a**–**b**

(1 mmol) and NaOH (10 mol%) in ethanol (10 mL). The mixture was heated under reflux for 2–2.5 h. and the progress of the reaction was monitored by TLC. After the completion of reaction (checked by TLC), the reaction mixture was cooled to room temperature and the solid separated was filtered and washed with equimolar mixture of chloroform and methanol to obtain the pure compounds **4a–r**. Analytical and spectroscopic characterization data of the selected compounds **4e** and **4q** are given below:

2.4a *1-Amino-3-[2-(4-methoxyphenyl)-1H-indol-3-yl]pyrido[1,2-a]benzimidazole-2,4-dicarbonitrile* **(4e)**: Dark yellow solid, yield 67%, m.p. 220°C; IR (KBr, ν, cm^{-1}): 3340 & 3210 (asym. & sym. str. of $-NH_2$), 2215 ($-C \equiv N$ str.), 1640 (C=N str.), 1575 (C=C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 3.76 (s, 3H, OCH_3), 6.98–8.54 (m, 12H, Ar−H), 8.56 (s, 2H, NH_2), 11.95 (s, 1H, indole NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 55.58 ($O\underline{C}H_3$), 79.17 (<u>C</u>-CN), 88.37 (<u>C</u>-CN), 105.95, 112.11, 114.88, 115.50, 116.22, 116.41, 119.20, 119.34, 120.59, 122.31, 122.59, 124.33, 127.01, 128.00, 129.06, 129.28, 136.40, 137.58, 145.25, 148.50, 149.16, 153.01, 159.72 (Ar−C); Mass (ESI-MS): m/z 454.7 $[M + H]^+$; Anal. Calcd. for $C_{28}H_{18}N_6O$ (454.15 g/mol): C, 74.00; H, 3.99; N, 18.49. Found: C, 73.84; H, 4.26; N, 18.29.

2.4b *1-Amino-3-[2-(4-isocyanophenyl)-1H-indol-3-yl]pyrido[1,2-a]benzimidazole-2,4-dicarbonitrile* **(4q)**: Yellow solid, yield 70%, m.p. 226°C; IR (KBr, ν, cm^{-1}): 3315 and 3240 (asym. and sym. str. of $-NH_2$), 2235 ($-C \equiv N$ str.), 1630 (C = N str.), 1575 (C = C str.); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H (ppm): 7.13–8.60 (m, 12H, Ar−H), 8.57 (s, 2H, NH_2), 11.97 (s, 1H, indole NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ_C (ppm): 79.25 (<u>C</u>-CN), 88.27 (<u>C</u>-CN), 107.86, 111.90, 115.33, 116.28, 116.73, 119.09, 119.45, 119.93, 121.25, 122.53, 124.29, 127.38, 128.01, 128.11, 128.59, 129.65, 133.16, 136.44, 137.20,

Table 1. Antimicrobial activity of the compounds **4a–r.**

			Minimum inhibitory concentration (MIC, μg/mL)							
			Gram-positive bacteria			Gram-negative bacteria			Fungi	
			S.P.	*C.T.*	*B.S.*	*S.T.*	*V.C.*	*E.C.*	*A.F.*	*C.A.*
Entry	R_1	R_2	MTCC 1936	MTCC 449	MTCC 441	MTCC 98	MTCC 3906	MTCC 443	MTCC 3008	MTCC 227
4a	H	H	200	**250**	**100**	250	**100**	200	>1000	**500**
4b	H	CH_3	250	**250**	**200**	250	125	250	>1000	1000
4c	CH_3	H	200	500	**100**	125	200	**62.5**	1000	1000
4d	CH_3	CH_3	**100**	500	**125**	200	250	200	500	>1000
4e	OCH_3	H	250	**250**	**125**	**50**	**100**	**25**	1000	**500**
4f	OCH_3	CH_3	250	**250**	500	250	200	**100**	>1000	1000
4g	Cl	H	500	**250**	**250**	500	125	250	>1000	1000
4h	Cl	CH_3	**100**	**200**	**125**	250	125	250	>1000	**250**
4i	Br	H	500	**125**	**100**	100	200	125	>1000	**500**
4j	Br	CH_3	125	**200**	**250**	200	125	200	1000	**500**
4k	F	H	200	**100**	**100**	125	200	**100**	1000	>1000
4l	F	CH_3	250	**200**	**125**	**62.5**	250	125	>1000	**250**
4m	SO_2CH_3	H	125	**250**	**200**	250	500	500	1000	**500**
4n	SO_2CH_3	CH_3	200	500	**125**	500	250	250	1000	1000
4o	NO_2	H	250	**200**	**100**	250	500	**100**	1000	1000
4p	NO_2	CH_3	**100**	**100**	**200**	200	200	200	1000	1000
4q	CN	H	125	**125**	**250**	**12.5**	**100**	**62.5**	1000	**500**
4r	CN	CH_3	250	**200**	**200**	200	125	200	500	>1000
	Ampicillin		100	250	250	100	100	100	–	–
	Norfloxacin		10	50	100	10	10	10	–	–
	Ciprofloxacin		50	100	50	25	25	25	–	–
	Chloramphenicol		50	50	50	50	50	50	–	–
	Griseofulvin		–	–	–	–	–	–	100	500
	Nystatin		–	–	–	–	–	–	100	100

Bold values indicate the active compounds
S.P., *Streptococcus pneumoniae*; C.T., *Clostridium tetani*; B.S., *Bacillus subtilis*; S.T., *Salmonella typhi*; V.C., *Vibrio cholerae*; E.C., *Escherichia coli*; A.F., *Aspergillus fumigatus*; C.A., *Candida albicans*
'–' represents 'not tested'

140.35, 145.39, 148.52, 148.93, 154.28 (Ar–C); Mass (ESI-MS): m/z 449.8 $[M + H]^+$. Anal. Calcd. for $C_{28}H_{15}N_7$ (449.14 g/mol): C, 74.82; H, 3.36; N, 21.81. Found: C, 74.64; H, 3.19; N, 21.93.

2.5 *Methodology for in vitro antimicrobial screening or minimal inhibitory concentration (MIC) measurement:*

The *in vitro* antimicrobial activity of all the compounds and standard drugs were assessed against three representative of Gram-positive bacteria viz. *Streptococcus pneumoniae* (MTCC 1936), *Clostridium tetani* (MTCC 449), *Bacillus subtilis* (MTCC 441), three Gram-negative bacteria viz. *Salmonella typhi* (MTCC 98), *Vibrio cholerae* (MTCC 3906), *Escherichia coli* (MTCC 443) and two fungi viz. *Aspergillus fumigatus* (MTCC 3008) *and Candida albicans* (MTCC 227) by the Broth Microdilution MIC method recommended by National Committee for Clinical Laboratory Standards (NCCLS).[24] The strains employed for the activity were procured from (MTCC — Micro Type Culture Collection) Institute of Microbial Technology, Chandigarh. Inoculum size for test strain was adjusted to 10^8 CFUmL^{-1} (Colony Forming Unit per milliliter) by comparing the turbidity (turbidimetric method). Mueller Hinton Broth was used as nutrient medium to grow and dilute the compound suspension for the test bacteria and Sabouraud Dextrose Broth used for fungal nutrition. Ampicillin, norfloxacin, ciprofloxacin and chloramphenicol were used as standard antibacterial drugs, whereas griseofulvin and nystatin were used as standard antifungal drugs. DMSO was used as diluents/vehicle to get desired concentration of synthesized compounds and standard drugs to test upon standard microbial strains. Serial dilutions were prepared in primary and secondary screening. Each compound and standard drugs were diluted obtaining 2000 μg/mL concentration, as a stock solution. In primary screening 1000, 500, and 250 μg/mL concentrations of the synthesized compounds were taken. The active compounds found in this primary screening were further diluted to obtain 200, 100, 62.5, 50, 25, 12.5 and 6.250 μg/mL concentrations for secondary screening to test in a second set of dilution against all microorganisms. The control tube containing no antibiotic is immediately sub cultured [before inoculation] by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism. The tubes are then put for incubation at 37°C for 24 h for bacteria and 48 h for fungi. The highest dilution (lowest concentration) preventing appearance of turbidity is considered as minimal inhibitory concentration (MIC, μg/mL) i.e., the amount of growth from the control tube before incubation (which represents the original inoculum) is compared. A set of tubes containing only seeded broth and the solvent controls were maintained under identical conditions so as to make sure that the solvent had no influence on strain growth. The result of this is much affected by the size of the inoculum. The test mixture should contain 10^8 CFUmL^{-1} organisms. The protocols were summarized in table 1 as the minimal inhibitory concentration (MIC, μg/mL).

2.6 *Methodology for in vitro antioxidant activity (FRAP assay)*

Ferric reducing antioxidant power (FRAP) was measured by a modified method of Benzie and Strain.[25] The antioxidant potentials of the compounds **4a–r** were estimated as their power to reduce the TPTZ-Fe(III) complex to TPTZ-Fe(II) complex (FRAP assay), which is simple, fast, and reproducible. FRAP working solution was prepared by mixing a 25.0 mL, 10 mM TPTZ solution in 40 mM HCl, 20 mM $FeCl_3 \cdot 6H_2O$ and 25 mL, 0.3 M acetate buffer at pH 3.6. A mixture of 40.0 μL, 0.5 mM sample solution and 1.2 mL FRAP reagent was incubated at 37°C for 15 min. Absorbance of intensive blue colour [Fe(II)-TPTZ] complex was measured at 593 nm. The ascorbic acid was used as a standard antioxidant compound. The results are expressed as ascorbic equivalent (mmol/100 g of dried compound).

3. Results and discussion

3.1 *Chemistry*

The required 2-phenyl-1*H*-indole-3-carboxaldehydes **1a–i** were prepared by Vilsmeier–Haack reaction of 2-phenyl-1*H*-indole according to literature procedure[26] and 2-cyanomethylbenzimidazoles **3a–b** were prepared according to literature procedure.[27]

In the present study, pyrido[1,2-*a*]benzimidazole derivatives **4a–r** (67–84%) (table 2) have been synthesized by cyclocondensation reaction of 2-phenyl-1*H*-indole-3-carboxaldehyde **1a–i**, malononitrile **2** and 2-cyanomethylbenzimidazole **3a–b** in the presence of catalytic amount of NaOH (scheme 1, table 3).

A variety of catalysts viz. piperidine,[28] piperidinium acetate,[29] pyridine,[30] NH_4OAc,[31] KOH[32] and $ZnCl_2$[33] have been used in the synthesis of pyrido[1,2-*a*]benzimidazole derivatives. We have also attempted this reaction without any catalyst as well as by using

Table 2. Substitution pattern of the compounds **4a–r.**

Entry	R_1	R_2	Yield[a](%)
4a	H	H	78
4b	H	CH_3	82
4c	CH_3	H	76
4d	CH_3	CH_3	80
4e	OCH_3	H	67
4f	OCH_3	CH_3	73
4g	Cl	H	82
4h	Cl	CH_3	84
4i	Br	H	78
4j	Br	CH_3	80
4k	F	H	73
4l	F	CH_3	76
4m	SO_2CH_3	H	70
4n	SO_2CH_3	CH_3	77
4o	NO_2	H	68
4p	NO_2	CH_3	72
4q	CN	H	70
4r	CN	CH_3	75

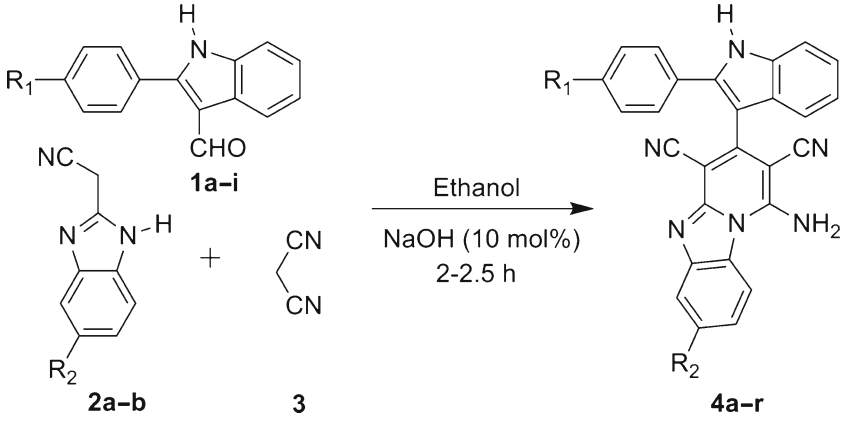
[a]Isolated yield of the pure compound

above catalysts. However, some shortcomings were observed in this method such as longer reaction time, poor yield, uncompletion of reaction and sticky materials have been observed. Interestingly, when the reaction was carried out in the presence of 10 mol% NaOH; it led to the desired product in 78% yield in 2.5 h. When we have performed this reaction in ehanol:water (1:1) by using 10 mol% NaOH as a catalyst, only Knoevenagel intermediate was formed without further cyclization while no reaction progress was observed in case of water as a solvent. The results are depicted in table 3. Here, we present a general, high yielding, non hazardous synthetic protocol by using NaOH as a catalyst.

In accordance with the mechanism suggested in literature,[34] the reaction occurs via an *in situ* initial formation of the indole-3-ylidenemalononitrile, containing the electron-poor C=C double bond, from the Knoevenagel condensation between 2-phenyl-1*H*-indole-3-carboxaldehydes **1a–i** and malononitrile **2** by loss of water molecule. Finally, Michael addition of 2-cyanomethylbenzimidazole **3a–b** to the initially formed unsaturated nitrile followed by cyclization and dehydrogenation to affords cyclized pyrido[1,2-*a*]benzimidazole **4a–r** (scheme 2).

The structures of all the newly synthesized compounds were confirmed by FTIR, ^{1}H NMR, ^{13}C NMR, mass and elemental analysis. In ^{1}H NMR (DMSO-d_6) spectrum of compound **4a–r**, the disappearance of a singlet from δ 10.20–10.70 ppm of -CHO clearly confirm the cyclization of Knoevenagel intermediate. Aromatic protons of **4a–r** resonate as multiplets in the range of δ 6.95–8.68 ppm and a singlet around δ 8.52–8.64 ppm exhibit NH_2 protons of the pyridine ring. The deshielded aromatic singlet around δ 11.95–12.17 ppm stands for secondary amine of the indole ring. ^{13}C NMR of **4a–r** exhibited a distinct signal around δ 78.95–79.28 ppm and δ 88.01–88.37 are assigned to two carbon attached with two carbonitrile while signals in the range of δ 105.95–159.72 ppm are attributed to all the aromatic carbon in the ^{13}C NMR spectra confirms the structure **4a–r**.

The IR spectrum of compound **4a–r** exhibited characteristic absorption band in the range of 3295–3345 and 3205–3240 cm^{-1} (asym. and sym. str.) for -NH_2 and 2205–2235 cm^{-1} for −CN functionality. The presence of band around 1625–1650 cm^{-1} of (C=N) and 1565–1585 cm^{-1} of alkene (C=C) functional group supports the formation of title compounds **4a–r**. Further, the structures of synthesized compounds were confirmed by its mass spectral studies. The mass spectra detected the expected molecular ion signals corresponding to respective molecular formula of synthesized compounds. The obtained elemental analysis values are in good agreement with theoretical data. All



Scheme 1. Synthetic pathway for the fused pyrido[1,2-*a*]benzimidazole derivatives **4a–r**.

Table 3. Influence of different catalysts for the reaction of pyrido[1,2-*a*]benzimidazole derivatives.

Entry	Catalyst	mol%	Solvent	Time (h)	Yield[a] (%)
1	–	–	Ethanol	7	0
2	Pyridine	10	Ethanol	6	30
3	Piperidine	10	Ethanol	5	55
4	Piperidinium acetate	10	Ethanol	7	19
5	NH_4OAc	10	Ethanol	5	0
6	KOH	10	Ethanol	4.5	36
7	$ZnCl_2$	10	Ethanol	5	0
8	NaOH	5	Ethanol	3	58
9	NaOH	10	Ethanol	2.5	78
10	NaOH	15	Ethanol	2.5	76
11	NaOH	20	Ethanol	2.5	50
12	NaOH	10	Ethanol + water (1:1)	7	0
13	NaOH	10	Water	7	0

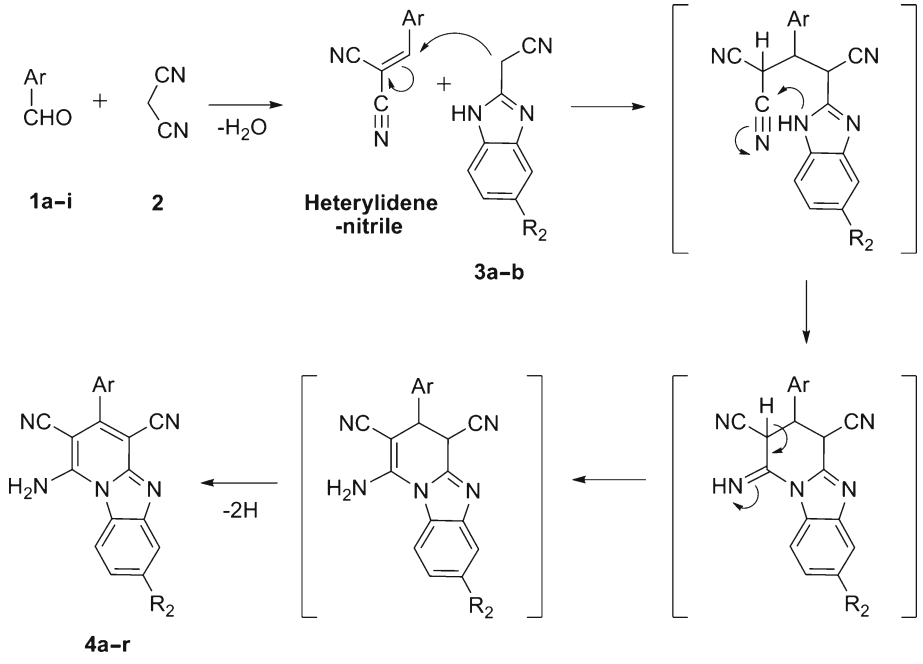
Molar ratio of starting materials (1:1:1) and reflux condition
[a]Isolated yield of the pure compound

spectroscopic and physiochemical data of compounds **4a–r** are given in section 2.

3.2 *Antimicrobial screening*

The examination of the antimicrobial activity data (table 1) reveals that most of the compounds showed effective antibacterial and antifungal activity against employed strains when compared with standard drugs ampicillin, norfloxacin, ciprofloxacin, chloramphenicol, griseofulvin and nystatin.

Upon investigation of antimicrobial activity data (table 1), it has been observed that compounds **4e** (MIC = 50 μg/mL), **4l** (MIC = 62.5 μg/mL) and **4q** (MIC = 12.5 μg/mL) showed excellent activity against Gram-negative bacteria *S. typhi*. as compared to ampicillin (MIC = 100 μg/mL), chloramphenicol (MIC = 50 μg/mL) and ciprofloxacin (MIC = 25 μg/mL). In case of *E. coli*, compounds **4e** (MIC = 25 μg/mL), **4c**



Scheme 2. Plausible mechanistic pathway for the synthesis of pyrido[1,2-*a*]benzimidazole derivatives.

and **4q** (MIC = 62.5 μg/mL) were found to be exceedingly potent upon comparison with ampicillin (MIC = 100 μg/mL), chloramphenicol (MIC = 50 μg/mL) and ciprofloxacin (MIC = 25 μg/mL). Compounds **4k**, **4p** (MIC = 100 μg/mL), **4i** and **4q** (MIC = 125 μg/mL) were shown outstanding activity as compared to the standard ampicillin (MIC = 250 μg/mL) as well as compounds **4h**, **4j**, **4l** and **4r** (MIC = 200 μg/mL) were found to be more active than ampicillin (MIC = 250 μg/mL) against *C. tetani*. As compared to ampicillin (MIC = 250 μg/mL), compounds **4a**, **4c**, **4i**, **4k**, **4o** (MIC = 100 μg/mL), **4d**, **4e**, **4h**, **4l** and **4m** (MIC = 125 μg/mL) were shown excellent activity as well as compounds **4b**, **4m**, **4p** and **4r** (MIC = 200 μg/mL) were found to possess better activity against *B. subtilis*. Whereas, against fungal pathogen *C. albicans*, compounds **4h** and **4l** (MIC = 4 250 μg/mL) displayed excellent activity upon comparison with griseofulvin (MIC = 500 μg/mL).

Compounds **4a**, **4b**, **4e**, **4f**, **4g** and **4m** were found to be equipotent to ampicillin (MIC = 250 μg/mL) as well as compounds **4k** and **4p** were equally active to ciprofloxacin (MIC = 100 μg/mL) in case of inhibiting *C. tetani*. Against *B. subtilis*, compounds **4g**, **4j** and **4q** displayed parallel results than that of ampicillin (MIC = 250 μg/mL), while compounds **4a**, **4c**, **4i**, **4k** and **4o** showed comparable activity to norfloxacin (MIC = 100 μg/mL) as well as compounds **4d**, **4h** and **4p** were found to possess similar activity to ampicillin (MIC = 100 μg/mL) towards *S. pneumoniae*.

Further, compounds **4a**, **4e** and **4q** against *V. cholerae* as well as compound **4i** against *S. typhi* were found equipotent to ampicillin (MIC = 100 μg/mL), while compounds **4f**, **4k** and **4o** were found equipotent as compared to ampicillin (MIC = 100 μg/mL) against *E. coli*. Whereas, compounds **4a**, **4e**, **4i**, **4j**, **4m** and **4q** were found to have equal activity with griseofulvin (MIC = 500 μg/mL) against *C. albicans*. Majority of the compounds were active against *B. subtilis* and *C. tetani*. Unfortunately, none of the synthesized compounds were found sufficiently potent to inhibit fungal pathogen *A. fumigatus*.

The investigation of the structure-activity relationship (figure 2) of antibacterial screening revealed that the majority of the compounds having R_2 = H showed excellent activity than R_2 = CH_3 against most of the employed strains. Compounds with 4-methoxy, cyano and unsubstituted phenyl ring at the 2-position of the indole nucleus carrying R_2 = H were found to be active against *C. tetani*, *B. subtilis*, *V. cholerae* and *C. albicans* as well as compounds with 4-methoxy, fluoro and nitro phenyl ring possessing R_2 = H displayed promising activity against *C. tetani*, *B. subtilis* and *E. coli*.

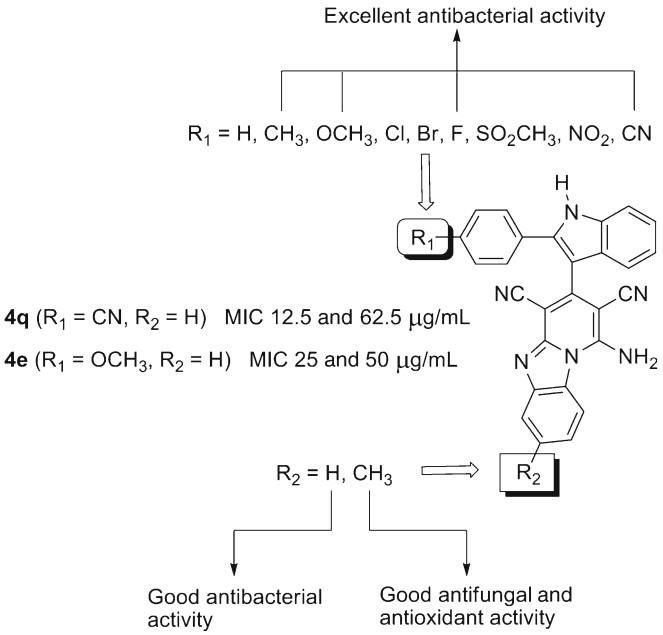


Figure 2. Schematic presentation of structure-activity relationship.

Compounds with 4-methyl, methoxy, methylsulphonyl, cyano and unsubstituted phenyl ring having R_2 = CH_3 were found to be less active against most of the bacterial and fungal pathogen.

Interestingly, compounds **4d**, **4h** and **4p** (R_2 = CH_3) were shown better activity against Gram-positive bacteria *S. pneumoniae*, while the reverse trend was observed in case of Gram-negative bacteria *V. cholerae*, compounds **4a**, **4e** and **4q** (R_2 = H) showed excellent activity compared to ampicillin. In case of inhibiting fungal pathogen, compounds **4h** and **4l** were found to be exceedingly potent towards *C. albicans* was observed possibly due to the presence of electron negative group on phenyl ring at 2-position of indole nucleus and R_2 = CH_3.

Moreover, compounds **4e** (R_1 = OCH_3, R_2 = H) and **4q** (R_1 = CN, R_2 = H) revealed outstanding inhibitory action against most of the representative panel of bacteria and fungi but upon replacing (R_2 = H) by (R_2 = CH_3), resulted compounds **4f** (R_1 = OCH_3, R_2 = CH_3) and **4q** (R_1 = CN, R_2 = CH_3) have been found totally inactive against most of the employed strains. Compound **4o** (R_1 = NO_2, R_2 = H) was found to have activity (MIC = 200 μg/mL) towards *C. tetani*, (MIC = 250 μg/mL) towards *S. typhi*, (MIC = 100 μg/mL) towards *E. coli*, (MIC = 500 μg/mL) towards *V. cholerae* and (MIC = 1000 μg/mL) towards *C. albicans* but upon replacing (R_1 = NO_2) by (R_1 = CN), resulted compound **4q** (R_1 = CN, R_2 = H) have been found to posses increased the potency (MIC = 125 μg/mL) against

Table 4. Antioxidant activity of the compounds **4a–r**.

Entry	FRAP value (mmol AA/100 g)	Entry	FRAP value (mmol AA/100 g)	Entry	FRAP value (mmol AA/100 g)
4a	94.5	**4g**	105.5	**4m**	87.9
4b	**123.7**	**4h**	**208.2**	**4n**	**120.3**
4c	106.1	**4i**	84.4	**4o**	109.0
4d	102.2	**4j**	117.9	**4p**	**209.9**
4e	102.7	**4k**	**125.7**	**4q**	115.7
4f	**128.4**	**4l**	**127.9**	**4r**	115.7

Bold values indicate the active compounds
AA = Ascorbic acid

C. tetani, (MIC = 12.5 μg/mL) against *S. typhi*, (MIC = 62.5 μg/mL) against *E. coli*, (MIC = 100 μg/mL) against *V. cholerae* and (MIC = 500 μg/mL) against *C. albicans*. Same thing was observed in case of compound **4c** (R_1 = CH_3, R_2 = H) have shown poor activity against most of the employed strains but upon replacing (R_1 = CH_3) by (R_1 = OCH_3), resulted increase in the potency of the compound **4e** (R_1 = OCH_3, R_2 = H) towards most of the employed strains.

Further, out of all the synthesized compounds, the **4e** (R_1 = OCH_3, R_2 = H) showed chief activity (MIC = 50 μg/mL) against *V. cholerae* and (MIC = 25 μg/mL) against *E. coli* as well as compound **4q** (R_1 = CN, R_2 = H) exhibited chief potency (MIC = 12.5 μg/mL) against *V. cholerae* than that of standard drugs.

3.3 *Antioxidant activity*

In vitro antioxidant power of all compounds was determined by FRAP assay. The FRAP value was expressed as an equivalent of standard antioxidant ascorbic acid (mmol/100 g of dried compound). FRAP values indicate that all the compounds have a ferric reducing antioxidant power. Compounds **4h** and **4p** showed relatively high antioxidant power while, compounds **4b**, **4f**, **4k**, **4l** and **4n** found to have better ferric reducing power. Compounds **4i** and **4m** displayed poor antioxidant potency (table 4). Interestingly, compounds **4e** and **4q** having (R_2 = H) were found to be exceedingly potent against most of the employed strains in antimicrobial screening while in antioxidant activity, highly efficient antioxidant members **4h** and **4p** possessing (R_2 = CH_3) substitution.

4. Conclusion

We report here the synthesis, antimicrobial and antioxidant activity of new pyrido[1,2-*a*]benzimidazole derivatives bearing indole nucleus. The engaged synthetic strategy efficiently involves the multicomponent reaction (MCR) approach, eco-friendly base catalysis and allows the construction of relatively complicated nitrogen containing heterocyclic system as well as the assimilation of two promising bioactive nuclei in a single scaffold through an easy way. Reviewing and comparing the activity data (tables 1 and 4), it is worthy to mention that the biological activity of the target compounds depends not only on the bicyclic heteroaromatic pharmacophore appended through aryl ring but also on the nature of the peripheral substituents and may also upon their spatial relationship and positional changes. Compounds **4c**, **4e**, **4l** and **4q** have found to be most efficient antimicrobial members while compounds **4h** and **4p** possess better ferric reducing antioxidant power. These studies can provide insight in defining the ample scope and boundaries of both allied candidates for further detailed pre-clinical investigations.

Supporting information

Analytical and spectroscopic characterization data of the synthesized compounds **4a–r** are available as supporting information in the Journal of Chemical Sciences website (www.ias.ac.in/chemsci).

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