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# Synthesis and *in vitro* antimicrobial screening of new pyrano[4,3-*b*]pyrane derivatives of 1*H*-pyrazole

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**Abstract**

A new series of pyrano[4,3-*b*]pyrane **4a–l** bearing 1*H*-pyrazole has been synthesized by one pot base catalyzed cyclocondensation reaction of 1*H*-pyrazole-4-carbaldehyde **1a–l**, malononitrile **2** and 4-hydroxy-6-methylpyrone **3**. All the synthesized compounds were screened against six bacterial pathogens, namely *B. subtilis*, *C. tetani*, *S. pneumoniae*, *S. typhi*, *V. cholerae*, *E. coli* and antifungal activity against, two fungal pathogens, *A. fumigatus* and *C. albicans* using broth microdilution MIC method. Some of the compounds are found to be equipotent or more potent than that of commercial drugs, against most of employed strains.


*Keywords:* Pyranopyrane; MCR; Pyrazole-4-carbaldehyde; Antimicrobial activity

An alarming increment in pathogenic resistance to existing first line standard drugs is a serious problem in antimicrobial cure and necessitates continuing research into new classes of antimicrobials [1]. Moreover, the progression of drug-resistant strains has contributed to the inefficiency of the straight antimicrobial therapy. This crop up an enormous interest in antibacterial research and we strongly believe that there is an urgent call for development of new drugs with divergent and unique structure and with a probably unusual mechanism of action from that of existing first line drugs. Consequently, this spot of research is accorded an immense significance and keeps on attracting much attention of increasing number of medicinal chemists.

Recently, we have been particularly interested in the synthesis of *N*-arylpyrazole and pyrane incorporating structures for antimicrobial evaluations [2] on the premise that *N*-arylpyrazole is chemically useful synthons bearing diverse biological activities like antimicrobial [3–5], anti-inflammatory (COX-2 inhibitor and ulcerogenic activity) [4], antitubercular [5], antitumor [6,7], antiangiogenesis [7], anti-parasitic [8], antiviral [9], analgesic and anxiolytic activity [10]. Moreover, the pyran nucleus is a fertile source of biologically important molecules possessing a wide spectrum of biological and pharmacological activities, such as antimicrobial [11], antiviral [12], antiproliferative [13], antitumor [14], *etc*.

Multi component reaction (MCR) approach emerged as the most suitable protocol for the synthesis of functionalized organic compounds due to the fact that the synthesis can be performed without the isolation of the intermediates, without discharging any functional groups within short reaction time [15]. This synthetic strategy is fruitfully employed to achieve new pyrano[4,3-*b*]pyran derivatives of aryloxypyrazole.

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Thus, in view of biological significance of pyrane, a modification on the 4-position on pyrano[4,3-*b*]pyrane by 3-methyl-5-phenoxy-1-phenyl-1*H*-pyrazole is undertaken to check whether it may bring significant changes in bioactivities of pyrane derivatives. As a part of our current studies in developing new antimicrobial agents *via* combination of two therapeutically active moieties [16], we have synthesized and report herein pyrano[4,3-*b*]pyrane derivatives **4a–l** *via* MCR approach.

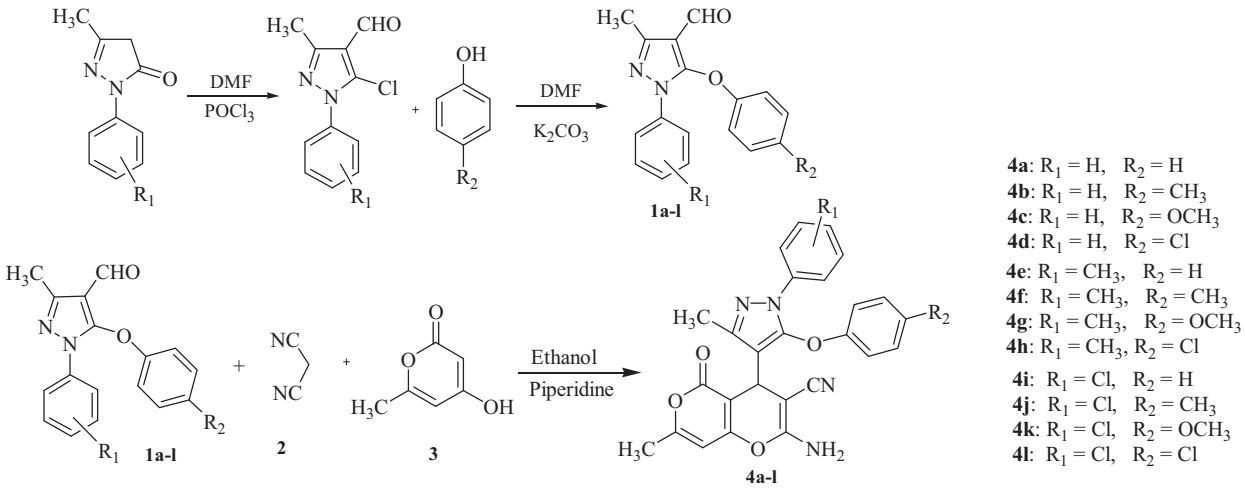
## 1. Experimental

All the reagents were obtained commercially and used with 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 60 $F_{254}$ (Merck). Elemental analysis (%C, H, N) was carried out by Perkin-Elmer 2400 series-II elemental analyzer (Perkin-Elmer, USA). 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 solvent peak as internal standard at 400 MHz and 100 MHz, respectively. Mass spectra were scanned on a Shimadzu LCMS 2010 spectrometer (Shimadzu, Tokyo, Japan) (Scheme 1).

### 1.1. General procedure

A mixture of an appropriate 1*H*-pyrazole-4-carbaldehyde **1a–l** (30 mmol), malononitrile **2** (30 mmol) and 4-hydroxy-6-methylpyrone **3** (30 mmol) in ethanol (20 mL) containing catalytic amount of piperidine was stirred under reflux for 1–1.5 h. On completion of reaction, monitored by TLC (ethyl acetate:hexane = 1:1), the reaction mixture was cooled to room temperature, the solid separated was filtered and washed well with ethanol to obtain the pure compounds **4a–l**. Physical, analytical and spectroscopic characterization data of the synthesized compounds **4a** and **4k** are given below:

*2-Amino-4-[3-methyl-5-phenoxy-1-phenyl-1H-pyrazol-4-yl]-7-methyl-5-oxo-4,5-dihydropyrano[4,3-b]pyran-3-carbonitrile* (**4a**): Yield: 80%; mp: 230–231 °C; IR (KBr, $\nu$, cm$^{-1}$): 3400 & 3180 (asym. & sym. str. of –$NH_2$), 2205 (–C≡N str.), 1710 (–C=O str.); $^1$H NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.00, 2.33 (s, 3H, 2 × $CH_3$), 4.28 (s, 1H, H4), 5.68 (s, 1H, H8), 6.57–7.54 (m, 12H, $NH_2$ + Ar–H). $^{13}$C NMR (100 MHz, DMSO-$d_6$): $\delta$ 13.07, 19.52 ($CH_3$), 25.88 (C4), 56.24 (C–CN), 97.95 (4a), 99.17 (C8), 110.82, 114.56, 120.03, 121.63, 123.38, 127.05, 129.63, 130.17, 138.09, 145.35, 148.07, 156.20, 158.36, 158.63, 161.82 (Ar–C), 162.55 (C=O); Anal. Calcd. for $C_{26}H_{20}N_4O_4$ (452.46): C, 69.02; H, 4.46; N, 12.38%. Found: C, 69.17; H, 4.35; N, 12.22%; MS *m/z*: 453.52 $[M+1]^+$.




Scheme 1. Synthetic pathway for the compounds **1a–l** and **4a–l**.

*2-Amino-4-[1-(3-chlorophenyl)-5-(4-methoxyphenoxy)-3-methyl-1H-pyrazol-4-yl]-7-methyl-5-oxo-4,5-dihydro-pyrano[4,3-b]pyran-3-carbonitrile* (**4k**): Yield: 84%; mp: 240–241 °C; IR (KBr, $\nu$, $cm^{-1}$): 3410 & 3195 (asym. & sym. str. of $–NH_2$), 2195 (–C≡N str.), 1705 (–C=O str.); $^1H$ NMR (400 MHz, DMSO-$d_6$): $\delta$ 2.03, 2.32 (s, 3H, 2 × $CH_3$), 3.66 (s, 3H, $OCH_3$), 4.28 (s, 1H, H4), 5.75 (s, 1H, H8), 6.53–7.61 (m, 10H, $NH_2$ + Ar–H). $^{13}C$ NMR (100 MHz, DMSO-$d_6$): $\delta$ 13.10, 19.41 ($CH_3$), 25.83 (C4), 55.82 ($OCH_3$), 56.01 (C–CN), 98.02 (4a), 99.04 (C8), 111.22, 115.20, 115.42, 119.57, 119.97, 120.92, 126.68, 131.42, 133.98, 139.22, 146.11, 148.86, 149.76, 155.52, 158.38, 158.63, 161.80 (Ar–C), 162.58 (C=O); Anal. Calcd. for $C_{27}H_{21}ClN_4O_5$ (516.93): C, 62.73; H, 4.09; N, 10.84%. Found: C, 62.95; H, 4.26; N, 10.99%; MS *m/z*: 517.88 $[M+1]^+$.

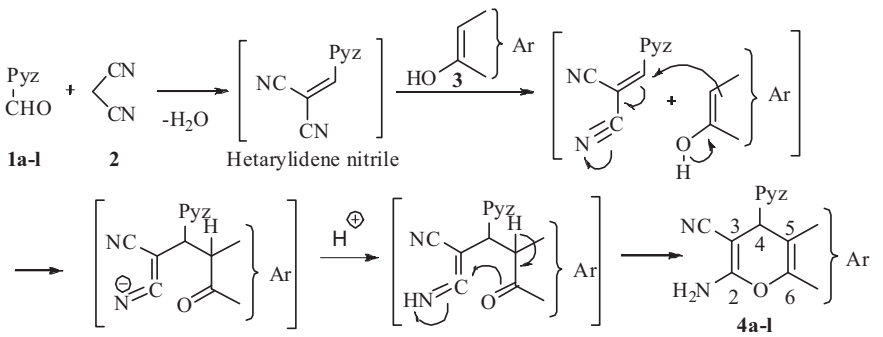
## 2. Results and discussion

The targeted title compounds pyrano[4,3-*b*]pyranes **4a–l** were prepared in moderate to good yield (75–90%) by the reaction of 3-methyl-5-aryloxy-1-aryl-1*H*-pyrazole-4-carbaldehydes, malononitrile and 4-hydroxy-6-methylpyrone in refluxing ethanol containing piperidine as a basic catalyst. The required 1-aryl-5-chloro-3-methyl-1*H*-pyrazole-4-carbaldehydes were prepared according to literature procedure [18] and 3-methyl-5-aryloxy-1-aryl-1*H*-pyrazole-4-carbaldehydes **1a–l** by our literature procedure [2a]. The reaction was carried out in aqueous media and under neutral conditions but failed to proceed even on prolong refluxing. The reaction was also attempted under microwave irradiation but not succeeded. The reaction proceeded in acetonitrile, methanol, benzene or DMF, in the presence of morpholine or $K_2CO_3$ but required prolong refluxing and resulted only in poor yield. Hence, these conditions were considered as the most optimized condition for the synthesis of title derivatives.

The plausible mechanism for the formation of the pyrane derivatives **4a–l** is outlined in Scheme 2. The reaction may proceed *via* an *in situ* initial formation of the hetarylidene nitrile, containing the electron-poor C=C double bond, from the Knoevenagel condensation between 3-methyl-5-aryloxy-1-arylpyrazole-4-carbaldehyde and malononitrile by loss of water molecules. Finally, Michael addition of **3** to the initially formed unsaturated nitrile, i.e. nucleophilic attack of hydroxyl moiety to the cyano olefins afforded cyclized pyrano[4,3-*b*]pyrane derivatives **4a–l**.

$^1H$ NMR (DMSO-$d_6$) spectrum of **4a** exhibited a singlet peak around $\delta$ 4.28, 5.68 stands for H4 and H8 respectively. Amine and aromatic protons of **4a** resonate as multiplets at around $\delta$ 6.57–7.54. Singlets around $\delta$ 2.00, 2.33 stands for methyl protons. $^{13}C$ NMR of **4a** exhibited distinctive signals around $\delta$ 13.07, 19.52 stands for methyl carbons and $\delta$ 25.88, 56.24 stands for C4 and C–CN respectively. Aromatic carbons of **4a** showed signals around $\delta$ 110.82–161.82 in the $^{13}C$ NMR spectra. Moreover, distinctive signals $\delta$ 162.55 stands for carbonyl carbon. The IR spectrum of compound **4a** exhibited characteristic absorption bands around 3400–3180 $cm^{-1}$ and 2205 $cm^{-1}$ stands for (asym. & sym. str.) $–NH_2$ and –CN functional groups respectively. The characteristic absorption band of lactone carbon is observed around 1710 $cm^{-1}$.

The elemental analysis values and mass spectral data are in good agreement with that of theoretical data. Similarly, all these compounds were characterized on the basis of spectral studies. All the compounds **4a–l** were screened for their antimicrobial activities using ampicillin, nystatin and griseofulvin as standard drugs by broth microdilution method as recommended by NCCLS [17]. Reviewing the antimicrobial activity data (Table 1), majority of compounds are found to be active against Gram-positive bacteria *B. subtilis*, *C. tetani* and a fungal pathogen *C. albicans*. It is worth mentioning that minor change in the molecular configuration of these compounds profoundly influences the activity.




Scheme 2. Plausible mechanistic pathway for the compounds **4a–l**.

Table 1
Antimicrobial activity of compounds **4a–l**.

| Compound | Minimum inhibitory concentration, MIC (μg/mL) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Gram-positive bacteria | | | Gram-negative bacteria | | | Fungal species | |
| | *B. subtilis* | *C. tetani* | *S. pneumoniae* | *E. coli* | *S. typhi* | *V. cholerae* | *A. fumigatus* | *C. albicans* |
| **4a** | 200 | 200 | 250 | 100 | 100 | 250 | 1000 | 500 |
| **4b** | 200 | 200 | 200 | 62.5 | 250 | 100 | 500 | 250 |
| **4c** | 250 | 500 | 250 | 500 | 500 | 500 | 500 | 250 |
| **4d** | 500 | 500 | 100 | 250 | 250 | 250 | 200 | 1000 |
| **4e** | 500 | 250 | 500 | 200 | 250 | 200 | 500 | 250 |
| **4f** | 100 | 250 | 500 | 250 | 500 | 250 | 1000 | 500 |
| **4g** | 200 | 250 | 200 | 200 | 250 | 200 | >1000 | 500 |
| **4h** | 500 | 200 | 100 | 500 | 500 | 250 | >1000 | 100 |
| **4i** | 500 | 250 | 250 | 500 | 500 | 500 | 500 | 250 |
| **4j** | 250 | 250 | 250 | 200 | 250 | 200 | 500 | 200 |
| **4k** | 250 | 250 | 500 | 62.5 | 100 | 100 | 1000 | 500 |
| **4l** | 200 | 500 | 200 | 500 | 200 | 500 | >1000 | 500 |
| A | 250 | 250 | 100 | 100 | 100 | 100 | – | – |
| B | – | – | – | – | – | – | 100 | 100 |
| C | – | – | – | – | – | – | 100 | 500 |

A, ampicillin; B, nystatin; C, griseofulvin; '–', 'not tested'.

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