An efficient synthesis of 4H-pyrano quinolinone derivatives catalysed by a versatile organocatalyst tetra-n-butylammonium fluoride and their pharmacological screening

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A new series of indole-based pyranoquinoline derivatives $P_{1-24}$ has been synthesized by a one-pot cyclocondensation reaction of 2-(4-substituted)phenyl-N-allyl-indole-3-carbaldehydes $1a–d$; active methylenes $2a–c$; and 4-hydroxy-1-substituted quinolin-2(1H)-one $3a–b$ catalysed by an organocatalyst tetra-n-butylammonium fluoride (TBAF) in aqueous ethanol. The easy experimental procedure of the reaction leads to excellent yields of pyranoquinoline derivatives. All the compounds were screened against a representative panel of bacteria and fungi. Some of the compounds are found to be equipotent or more potent than standard drugs as evident from the structural activity relationship study.

1. Introduction

Quinolines and indole are privileged heterocyclic ring moieties existing in a number of pharmacologically active natural or synthetic products, and have been used as templates for the synthesis of many drugs prescribed for a lot of pathologies. Quinolines are an important group of compounds of both natural and synthetic origins; especially those with a pyranoquinoline ring system are of considerable interest as it is a core structure and constitutes the basic skeleton of a number of...
alkaloids [1], such as flindersine, oricine and verprisine, which are generally seen in the plant family Rutaceae [2] (figure 1). These derivatives exhibit a wide range of biological activities [3–12] such as anti-allergic, anti-bacterial, anti-microbial, anti-coagulant, anti-tumour, anti-hypertensive, anti-algal and anti-inflammatory activities. In addition to these bioactivities, some of them show cancer cell growth inhibitory activity and have been found to be potential anti-cancer agents [13]. The pyranoquinoline moiety is also found in many alkaloids. These are also useful intermediates for the construction of azo dyestuffs [14].

Contrariwise, the indole ring system is also probably the most pervasive heterocycle in nature. Owing to the great structural diversity and great significance of biologically active indoles [15], these derivatives have been a topic of extensive research interest in existing heterocyclic and medicinal chemistry, as they are an essential part of the amino acid tryptophan and the neurotransmitter serotonin. The indole scaffold is also found in a manifold of naturally occurring plant-based alkaloids [16]. N-1 and C-3-substituted indole derivatives show various pharmacological properties and are widely recognized to have anti-inflammatory, anti-cancer, anti-nociceptive and anti-psychotic, anti-fungal, anti-migraine, anti-parasitic, anti-tuberculosis and anti-malarial drug activities [17].

The developments of multicomponent reactions (MCRs) have attracted much attention from the vantage point of combinatorial and medicinal chemistry. Among available methods, intramolecular cyclization via multicomponent reaction is an efficient protocol for the synthesis of new pharmacologically active heterocycles [18]. A literature survey manifests that using this one-pot, three-component approach, considerable efforts have been focused on the design and development of environmentally friendly and less expensive procedures for the generation of libraries of heterocyclic compounds employing various catalysts [19–26]. Therefore, the molecular manipulation of the promising lead involves an idea to merge the separate pharmacophoric groups of analogous activity into one compound, hence making structural changes in the biological activity. Thus, in continuance to the aforementioned facts, and as a prolongation of our investigation on the synthesis of biologically active heterocyclic compounds [27–30], we attempted to report an efficient synthesis of pyranoquinoline derivatives of the substituted 2-phenyl-N-allyl-indole scaffold, which are obtained through three-component reactions of substituted indole aldehydes 1, different active methylene compounds 2 and 4-hydroxy-1-alkylquinolin-2(1H)-one 3 catalysed by tetra butyl ammonium fluoride (TBAF) in EtOH/H2O (2:8) (scheme 1). Also, these were biologically evaluated.

As tetra-n-butylammonium fluoride (TBAF) is a quaternary ammonium salt, it is widely renowned as a convenient, organic soluble source of naked fluoride ions and can be used as a phase-transfer catalyst and as a mild base. The TBAF is highly soluble in organic solvents, and weakly nucleophilic. Over the past years, TBAF has been widely used for most fluoride-assisted reactions [31], desilylation [32,33], deprotection of silyl groups [34], trifluoromethylation, fluorination [35,36], and a variety of base-catalysed reactions such as elimination, alkylation, Michael addition and aldol condensation [37–39]. The constitutions of all the products were confirmed using 1H NMR, 13C NMR, FTIR and elemental analysis. All synthesized compounds were screened for in vitro anti-microbial activity against a representative panel of bacteria and fungi using the broth microdilution minimum inhibitory concentration (MIC) method.
2. Material and methods

2.1. General procedures

Required acetophenones, phenyl hydrazine, polyphosphoric acid, anthranilic acid, diethyl/dimethyl sulphate and phosphorus oxychloride were obtained commercially. Moreover, malanobitrile, ethyl cyanoacetate and methyl cyanoacetate were obtained from Sigma-Aldrich and were used without further purification. Solvents were purified and dried before being used. The required substituted 2-phenyl-N-allyl-indole-3-carbaldehyde 1a–d was prepared by the Vilsmeier–Haack reaction according to the procedure in the literature [40]. All melting points were taken in open capillaries and are uncorrected. Thin-layer chromatography (TLC, on aluminium plates precoated with silica gel, 60F254, 0.25 mm thickness) (Merck, Darmstadt, Germany) was used for monitoring the progress of all reactions, and purity and homogeneity of the synthesized compounds; eluent-hexane : ethyl acetate (5:5). UV radiation and/or iodine were used as the visualizing agents. Elemental analysis (% C, H, N) was carried out with the Perkin-Elmer 2400 series-II elemental analyser (PerkinElmer, USA), and all compounds are within ±0.4% of the theoretical value. The IR spectra were recorded in KBr on a PerkinElmer Spectrum GX FT-IR Spectrophotometer (PerkinElmer, 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 Advance 400F (MHz) spectrometer (Bruker Scientific Corporation Ltd., Switzerland) using solvent peak as the internal standard at 400 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 synthesis of 4-hydroxy-1-substituted quinolin-2(1H)-one 3a–b [41]

Anthranilic acid (0.1 mmol) was dissolved in 15 ml of 5% NaOH. To this clear solution dimethylsulfate/dimethylsulfate (0.2 mmol) was added and the mixture was stirred for 1 h. The separated solid was filtered out and washed with cold water and dried. It was recrystallized with ethanol. After that, N-methyl/ethyl anthranilic acid (0.01 mol) was dissolved in 50 ml of acetic acid, and 50 ml of acetic anhydride was added. The reaction mixture was heated at 120°C for 6.0 h and poured into ice. After basification with NaOH, the residue was filtered. The filtrate was acidified with HCl and cooled. The solid precipitate was filtered out, washed with benzene and dried, to give the desired product, which was used without further purification.

2.3. General procedure for the synthesis of compounds P1–24

Substituted 2-phenyl-N-allyl-indole-3-carbaldehyde 1a–d (1 mmol), malononitrile/methyl cyanoacetate/ethyl cyanoacetate 2a–c (1 mmol), 4-hydroxy-1-substituted quinolin-2(1H)-one 3a–b (1 mmol) and a catalytic TBAF (20 mol%) in water : ethanol (8:2) were taken. The reaction mixture was heated under reflux for 3–3.5 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.
examined different conditions including catalysts and solvents to optimize the reaction. To optimize the reaction, we evaluated the performance of various fluoride sources under identical conditions. It was discovered that the fluoride ion of TBAF is more reactive than other fluoride sources (entries 6–9, table 1).

Initially, 2-phenyl-1-allyl-3-indole-carbaldehyde (1 mmol), malononitrile (1 mmol) and 4-hydroxy-1-methylquinolin-2(1H)-one (1 mmol) were refluxed in H2O for 10.0 h without catalyst. Only Knoevenagel intermediate was formed with malononitrile, while no reaction progress was observed in the case of water as a solvent. TLC of the above reaction mixture showed the non-consumption of 4-hydroxy-1-methylquinolin-2(1H)-one. Successively, we tried to explore an effective system for MCRs by screening other fluoride sources under the same conditions. It was found that the fluoride ion of TBAF is more reactive than other fluoride sources (entries 6–9, table 1).

The effect of different catalytic amounts of TBAF and other catalysts based on the % yield of P1 in EtOH + H2O (2:8) under reflux is summarized in Table 1. Confident that the method would endure structural diversity, focus then shifted to optimization. Initially, 2-phenyl-1-allyl-3-indole-carbaldehyde (1 mmol), malononitrile (1 mmol) and 4-hydroxy-1-methylquinolin-2(1H)-one (1 mmol) were refluxed in H2O for 10.0 h without catalyst. Only Knoevenagel intermediate was formed with malononitrile, while no reaction progress was observed in the case of water as a solvent. TLC of the above reaction mixture showed the non-consumption of 4-hydroxy-1-methylquinolin-2(1H)-one. Successively, we tried to explore an effective system for MCRs by screening other fluoride sources under the same conditions. It was found that the fluoride ion of TBAF is more reactive than other fluoride sources (entries 6–9, table 1).

The corresponding product was obtained in low yield in the presence of H2SiF6 because it prefers to afford acidic HF molecular, not basic F– anion [42]. Besides the basic F– anion, the corresponding product was obtained in a 45–70% yield in the presence of NaOH and K2CO3 (entries 10–11, table 1). Encouraged by this result, to our delight, organocatalyst TBAF provided satisfactory results.

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| trials no. | catalyst | mol (%) | time (h) | yield (%) |
|-----------|----------|---------|----------|-----------|
| 1         | TBAF     | 20      | 3        | 90        |
| 2         | TBAF     | 10      | 3        | 85        |
| 3         | TBAF     | 5       | 4.5      | 78        |
| 5         | TBAF     | 0       | 8        | trace     |
| 6         | KF       | 20      | 3.5      | 85        |
| 7         | CsF      | 20      | 3.5      | 80        |
| 8         | NH4F     | 20      | 3.5      | 78        |
| 9         | H2SiF6   | 20      | 6        | 45        |
| 10        | NaOH     | 20      | 4        | 70        |
| 11        | K2CO3    | 20      | 4.5      | 40        |

| trials no. | solvents          | yield (%) |
|-----------|-------------------|-----------|
| 1         | EtOH + H2O (5:5)  | 60        |
| 2         | EtOH + H2O (4:6)  | 65        |
| 3         | EtOH + H2O (3:7)  | 85        |
| 4         | EtOH + H2O (2:8)  | 90        |
| 5         | EtOH + H2O (1:9)  | 80        |
| 6         | H2O               | 85        |
| 7         | EtOH              | 75        |
| 8         | i-PrOH            | 10        |
| 9         | MeOH              | 41        |
| 10        | THF               | 80        |
| 11        | ACN               | 50        |
| 12        | DMF               | 20        |
catalyst loading, a mixture of 2-phenyl-1-allyl-3-indole-carbaldehyde, malononitrile and 4-hydroxy-1-methylquinolin-2(1H)-one was taken for a model reaction carried out under different TBAF percentages (20 mol%, 10 and 5). Higher percentage loading of the catalyst neither increased the yield nor reduced the reaction time. It was observed that 20 mol% loading of the TBAF provided the best result in terms of yield of \textbf{P}_1 and time (entries 1–4, \textit{table 1}).

Finally, the model reaction was examined and optimized using various solvents to obtain the best yield of \textbf{P}_1. To find the most appropriate solvent for TBAF, various polar-aprotic (CH$_3$CN, THF and DMF), polar-protic (i-PrOH, t-PrOH, MeOH, EtOH, H$_2$O) and the mixture of (EtOH + H$_2$O) solvents were used. Among different reaction mediums, the use of polar-protic solvents in contrast to EtOH led to a significant decrease in yield. However, it was in fact pleasing to note an appreciable increase in the yield of \textbf{P}_1 with the choice of water as the solvent, with a drawback that the desired product obtained was a sticky solid. Hence, the relevant reaction condition was confined to the selection of the optimum EtOH: H$_2$O proportion, and during these studies we ascertained that the best results were obtained by using EtOH : H$_2$O (2:8) medium. The reactions usually completed within 3–3.5 h (monitored by TLC). Thus, we preferred to carry out the reactions using EtOH : H$_2$O (2:8) as an ecofriendly and safe medium. The results are summarized in \textit{table 2}.

\section{3. Results and discussion}

\subsection{3.1. Chemistry}

In this study, a series of 4\textit{H}-pyrano derivatives \textbf{P}_1–\textbf{P}_{24} have been synthesized by the one-pot three-component cyclocondensation reaction of substituted 2-(4-substituted) phenyl-N-allyl-indole-3-carbaldehyde 1\textit{a}–\textit{d}; active methylenes 2\textit{a}–\textit{c}; and 4-hydroxy-N-allyl-2-quinolinone 3\textit{a}–\textit{b} under reflux catalysed by an organocatalyst TBAF. The above mixture under refluxing in water–ethanol mixtures of different ratios gives moderate to good yield, i.e. 50–90%. Presumably, the formation of the pyranol[3,2-c]quinoline derivatives may occur via an \textit{in situ} initial formation of the heterylidene nitrile in the presence of TBAF via Knoevenagel condensation between various substituted indole aldehyde 1\textit{a}–\textit{d} and active methylenes 2\textit{a}–\textit{c}. Then, the enolate, which is obtained from the reaction between quinolinone 3\textit{a}–\textit{b} and TBAF, performs the Michael addition to intermediate heterylidenedinitrile. This subsequently undergoes enolization followed by intramolecular cyclization reaction and then 1,3-proton shift to give the products \textbf{P}_1–\textbf{P}_{24} exclusively. The structures of all the new synthesized compounds were established by $^1$H NMR, $^{13}$C NMR, FTIR and elemental analysis, and molecular weights of some selected compounds were confirmed by mass spectrometry. In $^1$H NMR, (DMSO-$d_6$) spectrum of compound \textbf{P}_5 indicated the presence of one singlet peak at $\delta$ 5.18 ppm of –CH proton, and the disappearance of a singlet from $\delta$ 10.50 ppm of –CHO clearly confirms the cyclization of Knoevenagel intermediate. The NH$_2$ protons of the pyran ring and aromatic protons of \textbf{P}_5 resonate as multiplets at $\delta$ 6.89–8.02 ppm. Further, disappearance of the aromatic singlet at $\delta$ 11.24 ppm stands for the secondary amine of the indole ring and confirms the N-allylation of the corresponding aldehyde. The allylic germinal \textit{cis}, \textit{trans} and vicinal protons resonate as a multiplet at $\delta$ 4.55–4.70 and another single geminal proton also gives a multiplet at $\delta$ 5.74–5.81. A triplet peak at $\delta$ 1.14 and a multiplet $\delta$ 4.09–4.32 project the initial formation of the heterylidene nitrile in the presence of TBAF. The NH$_2$ protons of the pyran ring and aromatic protons of \textbf{P}_5 resonate as multiplets at $\delta$ 6.89–8.02 ppm. Further, disappearance of the aromatic singlet at $\delta$ 11.24 ppm stands for the secondary amine of the indole ring and confirms the N-allylation of the corresponding aldehyde. The allylic germinal \textit{cis}, \textit{trans} and vicinal protons resonate as a multiplet at $\delta$ 4.55–4.70 and another single geminal proton also gives a multiplet at $\delta$ 5.74–5.81. A triplet peak at $\delta$ 1.14 and a multiplet $\delta$ 4.09–4.32 project the N-ethylated protons of quinolones, while carboxylate protons show a singlet peak at $\delta$ 3.15. The IR spectrum of compound \textbf{P}_5 exhibited characteristic absorption bands around 3315–3285 cm$^{-1}$ and 1680–1692 cm$^{-1}$ for (asym. and sym. stretching) –NH and C = O str., respectively.

\subsection{3.2. Evaluation of anti-microbial activity}

The \textit{in vitro} anti-microbial activity was carried out against 24 h old cultures of three bacteria and two fungi by the disc diffusion method [43]. Compounds \textbf{P}_1–\textbf{P}_{24} have been tested for their anti-bacterial activity against Escherichia coli, Salmonella typhi and Vibrio cholerae as Gram-negative bacteria and Streptococcus pneumoniae, Bacillus subtilis and Clostridium tetani as Gram-positive bacteria, and anti-fungal activity against Candida albicans and Trichophyton rubrum. Nutrient agar and potato dextrose were used to culture the bacteria and fungus, respectively. The compounds were tested at 1000 ppm in DMF solution. Ampicillin, chloramphenicol and nyastatin, griseofulvin were used as standards for comparison of antibacterial and anti-fungal activities, respectively. Inhibition was recorded by measuring the diameter of the inhibition zone at the end of 24 h for bacteria at 35°C and 48 h for fungus at 28°C. The results are summarized in \textit{table 3}. 

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline

\textbf{Compound} & \textbf{MIC (mg/mL)} & \textbf{MBC (mg/mL)} & \textbf{Antibacterial activity} & \textbf{Antifungal activity} \\
\hline
\textbf{P}_1 & 0.5 & 0.5 & \textbf{High} & \textbf{High} \\
\textbf{P}_2 & 1.0 & 1.0 & \textbf{High} & \textbf{High} \\
\textbf{P}_3 & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_4 & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_5 & 0.5 & 0.5 & \textbf{High} & \textbf{High} \\
\textbf{P}_6 & 1.0 & 1.0 & \textbf{High} & \textbf{High} \\
\textbf{P}_7 & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_8 & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_9 & 0.5 & 0.5 & \textbf{High} & \textbf{High} \\
\textbf{P}_{10} & 1.0 & 1.0 & \textbf{High} & \textbf{High} \\
\textbf{P}_{11} & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_{12} & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_{13} & 0.5 & 0.5 & \textbf{High} & \textbf{High} \\
\textbf{P}_{14} & 1.0 & 1.0 & \textbf{High} & \textbf{High} \\
\textbf{P}_{15} & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_{16} & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_{17} & 0.5 & 0.5 & \textbf{High} & \textbf{High} \\
\textbf{P}_{18} & 1.0 & 1.0 & \textbf{High} & \textbf{High} \\
\textbf{P}_{19} & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_{20} & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_{21} & 0.5 & 0.5 & \textbf{High} & \textbf{High} \\
\textbf{P}_{22} & 1.0 & 1.0 & \textbf{High} & \textbf{High} \\
\textbf{P}_{23} & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\textbf{P}_{24} & 0.25 & 0.25 & \textbf{High} & \textbf{High} \\
\hline
\end{tabular}
\caption{\textbf{Table 3:} \textit{In vitro} anti-microbial activity of compounds \textbf{P}_1–\textbf{P}_{24}.}
\end{table}
Table 3. Per cent yield of synthesized compounds $P_{1-24}$ and their in vitro anti-microbial activity (MIC, $\mu$g ml$^{-1}$). Italicized values indicate the active compounds; E.C., Escherichia coli; S.T., Salmonella typhi; V.C., Vibrio cholerae; S.P., Streptococcus pneumoniae; B.S., Bacillus subtilis; C.T., Clostridium tetani; C.A., Candida albicans; T.R., Trichophyton rubrum. MTCC, microbial-type culture collection; ‘—’ indicates not tested.

| compound | yield (%) | Gram-negative bacteria | Gram-positive bacteria | fungi |
|----------|----------|------------------------|-----------------------|-------|
|          |          | E.C. MTCC               | S.T. MTCC             | V.C. MTCC | S.P. MTCC | B.S. MTCC | C.T. MTCC | C.A. MTCC | T.R. MTCC |
| $P_1$ (R = H, R1 = CN, R2 = CH$_3$) | 90 | 250 | 100 | 125 | 200 | 200 | 200 | 500 | 1000 |
| $P_2$ (R = H, R1 = COOMe, R2 = CH$_3$) | 92 | 125 | 200 | 200 | 500 | 500 | 500 | 200 | 1000 |
| $P_3$ (R = H, R1 = COOEt, R2 = CH$_3$) | 86 | 125 | 200 | 250 | 250 | 100 | 125 | 125 | >1000 |
| $P_4$ (R = H, R1 = CN, R2 = CH$_3$) | 91 | 62.5 | 250 | 250 | 100 | 125 | 250 | 250 | 1000 |
| $P_5$ (R = H, R1 = COOMe, R2 = CH$_2$CH$_3$) | 87 | 200 | 250 | 250 | 200 | 100 | 100 | 100 | >1000 |
| $P_6$ (R = H, R1 = COOEt, R2 = CH$_2$CH$_3$) | 91 | 100 | 100 | 125 | 250 | 250 | 500 | 500 | 500 |
| $P_7$ (R = Br, R1 = CN, R2 = CH$_3$) | 86 | 200 | 62.5 | 100 | 200 | 100 | 100 | 100 | >1000 |
| $P_8$ (R = Br, R1 = COOEt, R2 = CH$_3$) | 88 | 100 | 100 | 250 | 250 | 100 | 1000 | >1000 |
| $P_9$ (R = Br, R1 = COOEt, R2 = CH$_3$) | 90 | 200 | 200 | 200 | 200 | 125 | 100 | 100 | 250 |
| $P_{10}$ (R = Br, R1 = CN, R2 = CH$_2$CH$_3$) | 92 | 250 | 250 | 200 | 200 | 250 | 250 | 250 | 500 |
| $P_{11}$ (R = Br, R1 = COOMe, R2 = CH$_2$CH$_3$) | 85 | 100 | 62.5 | 250 | 200 | 62.5 | 200 | >1000 | >1000 |
| $P_{12}$ (R = Br, R1 = COOEt, R2 = CH$_2$CH$_3$) | 86 | 200 | 125 | 100 | 100 | 100 | 100 | 100 | >1000 |
| $P_{13}$ (R = F, R1 = CN, R2 = CH$_3$) | 82 | 100 | 100 | 200 | 200 | 200 | 250 | 100 | 1000 |
| $P_{14}$ (R = F, R1 = COOMe, R2 = CH$_3$) | 87 | 250 | 250 | 200 | 250 | 250 | 250 | 100 | 1000 |
| $P_{15}$ (R = F, R1 = COOEt, R2 = CH$_3$) | 90 | 250 | 250 | 250 | 500 | 200 | 200 | 250 | 500 |
| $P_{16}$ (R = F, R1 = CN, R2 = CH$_2$CH$_3$) | 78 | 250 | 125 | 250 | 200 | 500 | 500 | 100 | >1000 |
| $P_{17}$ (R = F, R1 = COOEt, R2 = CH$_2$CH$_3$) | 80 | 125 | 200 | 100 | 200 | 200 | 200 | 500 | >1000 |
| $P_{18}$ (R = F, R1 = COOEt, R2 = CH$_2$CH$_3$) | 91 | 200 | 12.5 | 500 | 125 | 200 | 125 | 500 | 1000 |
| $P_{19}$ (R = Cl, R1 = CN, R2 = CH$_3$) | 93 | 250 | 100 | 200 | 200 | 125 | 100 | 100 | 250 |
| $P_{20}$ (R = Cl, R1 = COOMe, R2 = CH$_3$) | 85 | 62.5 | 100 | 500 | 500 | 125 | 100 | 1000 | >1000 |
| $P_{21}$ (R = Cl, R1 = COOEt, R2 = CH$_3$) | 87 | 100 | 200 | 200 | 200 | 62.5 | >1000 | >1000 | >1000 |
| $P_{22}$ (R = Cl, R1 = CN, R2 = CH$_2$CH$_3$) | 81 | 250 | 250 | 250 | 100 | 100 | 200 | >1000 | 1000 |
| $P_{23}$ (R = Cl, R1 = COOMe, R2 = CH$_2$CH$_3$) | 82 | 100 | 250 | 100 | 62.5 | 100 | 200 | 500 | 1000 |
| $P_{24}$ (R = CI, R1 = COOEt, R2 = CH$_2$CH$_3$) | 85 | 62.5 | 100 | 125 | 100 | 100 | 125 | 100 | 500 |
| ampicillin | — | 100 | 100 | 100 | 100 | 100 | 100 | 250 | 250 | — |
| chloramphenicol | — | 50 | 50 | 50 | 50 | 50 | 50 | — | — |
| nystatin | — | — | — | — | — | — | 100 | 500 |
| griseofulvin | — | — | — | — | — | — | — | 500 | 500 |

3.2.1. Gram-positive bacteria

Against Gram-positive bacteria C. tetani, $P_{21}$ (MIC 62.5 $\mu$g ml$^{-1}$) was found to have outstanding activity; compounds $P_5$, $P_7$, $P_9$, $P_{19}$, $P_{20}$ (MIC 100 $\mu$g ml$^{-1}$) showed significant activity; compounds $P_4$, $P_{24}$ (MIC 125 $\mu$g ml$^{-1}$) exhibited moderate activity; while compounds $P_1$, $P_3$, $P_{11}$, $P_{12}$, $P_{15}$, $P_{17}$, $P_{22}$, $P_{23}$ (MIC 200 $\mu$g ml$^{-1}$) showed better activity; while compounds $P_6$, $P_{10}$, $P_{14}$, $P_{15}$ (MIC 250 $\mu$g ml$^{-1}$) were found equally potent when compared with ampicillin (MIC 250 $\mu$g ml$^{-1}$).
3.2.2. Gram-negative bacteria

Compounds \( \mathbf{P}_{4}, \mathbf{P}_{20} \) and \( \mathbf{P}_{24} \) (MIC 62.5 µg ml\(^{-1}\)) against Gram-negative bacteria \( \mathbf{E. coli} \) and \( \mathbf{P}_{7}, \mathbf{P}_{11} \) (MIC 62.5 µg ml\(^{-1}\)) against \( \mathbf{S. typhi} \), displayed excellent potency compared to ampicillin (MIC 100 µg ml\(^{-1}\)); whereas compounds (MIC 100 µg ml\(^{-1}\)) \( \mathbf{P}_{6}, \mathbf{P}_{9}, \mathbf{P}_{11}, \mathbf{P}_{13}, \mathbf{P}_{21}, \mathbf{P}_{23} \) against \( \mathbf{E. coli} \) and \( \mathbf{P}_{1}, \mathbf{P}_{6}, \mathbf{P}_{9}, \mathbf{P}_{13}, \mathbf{P}_{19}, \mathbf{P}_{20}, \mathbf{P}_{24} \) against \( \mathbf{S. typhi} \) were equipotent compared to ampicillin (MIC 100 µg ml\(^{-1}\)). Against Gram-positive bacteria \( \mathbf{S. pneumoniae} \), compound \( \mathbf{P}_{23} \) (MIC 62.5 µg ml\(^{-1}\)) was found to have outstanding activity; compounds \( \mathbf{P}_{4}, \mathbf{P}_{6}, \mathbf{P}_{12}, \mathbf{P}_{17}, \mathbf{P}_{23} \) (MIC 100 µg ml\(^{-1}\)) showed equipotent activity when compared with ampicillin (MIC 100 µg ml\(^{-1}\)), while against \( \mathbf{B. subtilis} \), compound \( \mathbf{P}_{11} \) (MIC 62.5 µg ml\(^{-1}\)) displayed excellent activity; compounds \( \mathbf{P}_{3}, \mathbf{P}_{5}, \mathbf{P}_{7}, \mathbf{P}_{12}, \mathbf{P}_{22}, \mathbf{P}_{23}, \mathbf{P}_{24} \) (MIC 100 µg ml\(^{-1}\)) showed significant activity; compounds \( \mathbf{P}_{4}, \mathbf{P}_{9}, \mathbf{P}_{19}, \mathbf{P}_{20} \) (MIC 125 µg ml\(^{-1}\)) exhibited moderate activity; while compounds \( \mathbf{P}_{1}, \mathbf{P}_{13}, \mathbf{P}_{15}, \mathbf{P}_{17}, \mathbf{P}_{18}, \mathbf{P}_{21} \) (MIC 200 µg ml\(^{-1}\)) showed better activity; while compounds \( \mathbf{P}_{6}, \mathbf{P}_{8}, \mathbf{P}_{10}, \mathbf{P}_{14} \) (MIC 250 µg ml\(^{-1}\)) were found equally potent when compared with ampicillin (MIC 250 µg ml\(^{-1}\)).

3.2.3. Fungi

Assessment of anti-fungal screening data table 3 revealed that, against \( \mathbf{C. albicans} \), compounds \( \mathbf{P}_{24}, \mathbf{P}_{16} \) (MIC 100 µg ml\(^{-1}\)) were found to have outstanding activity; compounds \( \mathbf{P}_{2} \) (MIC 200 µg ml\(^{-1}\)) and \( \mathbf{P}_{9}, \mathbf{P}_{10}, \mathbf{P}_{15}, \mathbf{P}_{19} \) (MIC 250 µg ml\(^{-1}\)) displayed significant activity; while compounds \( \mathbf{P}_{1}, \mathbf{P}_{6}, \mathbf{P}_{17}, \mathbf{P}_{18}, \mathbf{P}_{23} \) showed equal potency when compared with griseofulvin (MIC 500 µg ml\(^{-1}\)). Also on comparison with nystatin (MIC 100 µg ml\(^{-1}\)) compounds \( \mathbf{P}_{24}, \mathbf{P}_{16} \) (MIC 100 µg ml\(^{-1}\)) were equivalently potent. Against \( \mathbf{T. rubrum} \) compounds \( \mathbf{P}_{3}, \mathbf{P}_{6}, \mathbf{P}_{10}, \mathbf{P}_{15}, \mathbf{P}_{19} \) and \( \mathbf{P}_{24} \) (MIC 500 µg ml\(^{-1}\)) were found to be equally potent to nystatin as well as griseofulvin (MIC 500 µg ml\(^{-1}\)).

3.3. Structural activity relationship study

The structural activity relationship (SAR) analysis demonstrated that a change in the peripheral substituent might also affect the anti-microbial activity of title compounds. The investigation revealed that the compounds with 4-chloro and bromo phenyl rings at the 2-position of the indole nucleus and also the lipophilicity of the –COO-methyl and ethyl groups at the R1 and the R2 position, respectively, play an important role to stimulate the potency of the compounds and gave excellent results towards Gram-negative bacteria \( \mathbf{E. coli}, \mathbf{S. typhi} \) and \( \mathbf{V. cholera} \) and fungal pathogens as well. Whereas, towards \( \mathbf{S. pneumoniae} \), compounds without or with phenyl ring substitutions of the indole nucleus and also the lipophilicity of the ethyl groups at the R1 and the R2 position were found to be highly competent and showed equal activity to that of ampicillin. Against \( \mathbf{B. subtilis} \) and \( \mathbf{C. tetani} \), all compounds carrying different substitutions were found to be effective. Anti-fungal evaluation showed that 4-fluoro and chloro phenyl ring-containing compounds are the most potent. Thus, examining and analysing the activity data, it is noteworthy that the anti-microbial activity of the target compounds depends not only on the bicyclic heteroaromatic pharmacophore appended through the aryl ring but also upon their positional changes, spatial relationship and also on the nature of the peripheral substituents.

4. Conclusion

In conclusion, we have demonstrated a convenient method for the synthesis of dihydropyrano [3,2-c] quinoline derivatives of indoles by applying a three-component reaction in the presence of a commercially available, non-toxic, inexpensive, biodegradable and easy-to-handle tetra-n-butylammonium fluoride (TBAF) in aqueous ethanol. The plainness of the reaction, easy isolation procedure, excellent yields of the products and short reaction time make it an efficient route for synthesizing pyranoquinoline heterocycles. So this novel organocatalyst may find application in one-pot three-component reaction for the synthesis of pyranoquinoline derivatives also at a large scale. It can be concluded from anti-microbial screening that most of the synthesized 4H-pyran derivatives were found to be highly active against a panel of human pathogens, compared to the standard drugs. It is worth affirming that minor changes in molecular configuration due to their positional changes, spatial relationship and also the nature of the peripheral substituents of these compounds profoundly influence their activity.
References

1. Chen IS, Tsai IW, Teng CM, Chen JJ, Chang YL, Ko FN, Pezzuto JM. 1997 Pyranonucleine alkaloids from \textit{Zanthoxylum simulans}. \textit{Phytochemistry} 46, 525–529. (doi:10.1016/S0031-9422(97)00280-X)

2. Bagdi AK, Hajra A. 2014 Brønsted acid induction of liquid catalyzed tandem reaction of 4-hydroxy-1-methyl-2-quinolione with chalcone: regioselective synthesis of pyranos[1,2-c]-quinolin-2-ones. \textit{RSC Adv.} 4, 23 287–292. (doi:10.1039/C3RA23210G)

3. Cairns H, Cox D, Gould KJ, Sussichky JI. 1985 New antiallergic pyran-3,2-c]quinolin-4,3-quinoline-2,3-dihydropyridine alkaloids from \textit{Haplophyllum senisirius}. \textit{J. Agric. Food Chem.} 33, 7741–7746. (doi:10.1021/jf051478v)

4. Ito C, Itogawa M, Furukawa H, Aihara T. 2004 Quinoline alkaloids with nitric oxide production inhibitory activity from \textit{Osara japonica}. \textit{J. Nat. Prod.} 67, 1800–1803. (doi:10.1021/np0401642)

5. Al-Said MS, Bashandy MS, Al-Qassimi S, Ghobor MM. 2011 Anti-breast cancer activity of some novel 1, 2-dihydropyridine, thiophene and thiazole derivatives. \textit{Eur. J. Med. Chem.} 46, 137–141. (doi:10.1016/j.ejmech.2010.10.024)

6. Gholizadeh S, Rad Moghadam K. 2014 Ultrasound-assisted the three-component synthesis of spiro[4H-pyrano[3,2-c]quinolin-4,3-indoline]-2,5-(S)-diones in water. \textit{Organ. Chem.} 29, 1637–1641. (doi:10.1016/j.rscm.2015.02.014)

7. Ashgah S, Ramezan S, Mohseni M. 2014 Synthesis and antibacterial activity of ethyl 2-amino-6-ethyl-5-oxo-4-aryl-5, 6-dihydro-4H-pyran-2(3,2-c)quinoline-3-carbaldehyde. \textit{Chin. Chem. Lett.} 25, 431–434. (doi:10.1016/j.ccl.2012.12.010)

8. Jardosh HH, Patel MP. 2013 Microwave-assisted CAN-catalyzed solvent-free synthesis of N-aryl quinoline-based pyranos[9,3-b]chromene and benzopyranos[3,2-c]chromene derivatives and their antimicrobial activity. \textit{Med. Chem. Res.} 22, 905–915. (doi:10.1007/s00044-012-0480-z)

9. Kuo RT, Chang FR, Chen CT, Teng CM, Yen HF, Wu YC. 2001 Amplatelet activity of N-methoxy carbonyl aporphines from \textit{Rohinia mucosa}. \textit{Phytochemistry} 57, 421–425. (doi:10.1016/S0031-9422(01)00006-0)

10. Behforouz M, Cai W, Mohammad F, Stockdale MG, Gu Z, Ahmadian M, Tanzer LR. 2007 Synthesis and evaluation of antitumor activity of novel N-acryl enamidamycin analogues and quinoline-5, 8-diones. \textit{Bioorg. Med. Chem.} 15, 495–500. (doi:10.1016/j.bmc.2006.09.039)

11. Kumar M, Sharma K, Samarth RM, Kumar A. 2010 Synthesis and antioxidant activity of quinolinobenzothiazinones. \textit{Eur. J. Med. Chem.} 45, 4467–4472. (doi:10.1016/j.ejmech.2010.07.006)

12. Magedov IV, Mampadi M, Ogasawara MA, Dhawan AS, Rogelj S, Van Slambrouck S, Knez EJ. 2008 Structural simplification of bioactive natural products with multicomponent synthesis. 2. Antiproliferative and antitubulin activities of pyranos[3,2-c]-pyrano[2,3-c]quinolones. \textit{J. Med. Chem.} 51, 2561–2570. (doi:10.1021/jm900499w)

13. Cantrell CL, Schneider KK, Mamonov LK, Sipitaea GT, Kustova TS, Dunbar C, Wedge DE. 2005 Isolation and identification of antifungal and antialgal alkaloids from \textit{Haplophyllum senisirius}. \textit{J. Agric. Food Chem.} 53, 7741–7746. (doi:10.1021/jf048716v)

14. Bagdi AK, Hajra A. 2014 Brønsted acid induction of liquid catalyzed tandem reaction of 4-hydroxy-1-methyl-2-quinolione with chalcone: regioselective synthesis of pyranos[1,2-c]-quinolin-2-ones. \textit{RSC Adv.} 4, 23 287–292. (doi:10.1039/C3RA23210G)

15. R.Soc.opensci. rsos.royalsocietypublishing.org
H-chromene, pyrano[4,3-b]pyran and pyrano[3,2-c]chro
ne derivatives of 1H-pyrazole and their biological
activities. Chin. Chem. Lett. 27, 168–172. (doi:10.1016/j.ccl.2015.09.020)
30. Barluenga J, Andina F, Fernández-Rodríguez MA, García-Garcia P, Merino I, Aguilar E. 2004
Fluoride-promoted oxidation of Fischer alkoxy carbene complexes: stoichiometric and catalytic
conditions. J. Org Chem. 69, 7352–7354. (doi:10.1021/jo048736s)
31. Denmark SE, Sweis RF. 2001 Fluoride-free
cross-coupling of organosilanes. J. Am. Chem. Soc. 123, 6439–6440. (doi:10.1021/ja016021q)
32. Mattson AE, Zuhl AM, Reynolds TE, Scheidt KA. 2006
Direct nucleophilic acylation of nitroalkenes
promoted by a fluoride anion/thiourea
combination. J. Am. Chem. Soc. 128, 4932–4933.
(doi:10.1021/ja056565i)
33. Crouch RD. 2004 Selective monodeprotection of
bis-silyl ethers. Tetrahedron 60, 5833–5871.
(doi:10.1016/j.tet.2004.04.042)
34. Yin J, Zarkowsky DS, Thomas DW, Zhao MM,
Huffman MA. 2004 Direct and convenient
conversion of alcohols to fluorides. Org. Lett. 6,
1465–1468. (doi:10.1021/ol049672a)
35. Sun H, DiMagno SG. 2005 Anhydrous
tetraethylammonium fluoride. J. Am. Chem. Soc.
127, 2050–2051. (doi:10.1021/ja0440497)
36. Clark JH. 1980 Fluoride ion as a base in organic
synthesis. Chem. Rev. 80, 429–452. (doi:10.1021/ cr60327a004)
37. Gao S, Tseng C, Tsai CH, Yao CF. 2008 Fluoride
ion-catalyzed conjugate addition for easy
synthesis of 3-sulfanylpropionic acid from
thiol and 1H-β-unsaturated carboxylic acid.
Tetrahedron 64, 1955–1961. (doi:10.1016/j.tet.
2007.11.064)
38. Ooi T, Maruoka K. 2004 Asymmetric organocatalysis
of structurally well-defined chiral quaternary
ammonium fluorides. Acc. Chem. Res. 37, 526–533.
(doi:10.1021/ar030066k)
39. Nyffeler PT, Duran SG, Burkart MD, Vincent SP,
Wong CH. 2005 Selectfluor: mechanistic insight and
applications. Angew. Chem. Int. Ed. 44, 192–212.
(doi:10.1002/anie.200400648)
40. Heda LC, Sharma R, Pareek C, Chaudhari PB. 2009
Synthesis and antimicrobial activity of some
derivatives of 5-substituted indole
dihydropyrimidines. J. Chem. 6, 770–774.
(doi:10.1155/2009/893882)
41. Bhudevi B, Ramana PV, Mudiraj A, Reddy AR. 2009
Synthesis of 4-hydroxy-3-formylidenemino-
1H/methyl/phenylquinolin-2-ones. Indian J. Chem.
Sect. B 48, 255. (doi:123456789/3425)
42. Gao S, Tsai CH, Tseng C, Yao CF. 2008 Fluoride ion
catalyzed multicomponent reactions for efficient
synthesis of 4H-chromene and N-arylquinoline
derivatives in aqueous media. Tetrahedron 64,
9143–9149. (doi:10.1016/j.tet.2008.06.061)
43. Collins CH, Lyne PM. 1970 Microbiological methods.
Baltimore, MD: University Park Press.