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Synthesis and Biological Screening of Pyrano[2,3-*b*]quinoline Derivatives

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Herein, a novel ionic liquid catalyzed synthesis of 3-acetylpyrano[2,3-*b*]quinolin-2(1*H*)-ones (**3a-e**) from substituted 3-formylquinolin-2(1*H*)-ones (**1a-e**) and ethyl acetoacetate (**2**) through Knoevenagel condensation is reported. We have perceived the application of microwave irradiation and ionic liquid for carrying out pollution free and ecofriendly chemical reactions. These reactions proceeded much faster in ionic liquid medium under microwave irradiation. The structures of quinoline derivatives (**3a-e**) were characterized by standard physico-chemical techniques. The synthesized quinoline derivatives (**3a-e**) were also evaluated for their *in vitro* antimicrobial screening on different strains of bacteria and fungi.

Keywords: Microwave synthesis, Ionic Liquid, Quinolines, Knoevenagel condensation, Antimicrobial activity.

INTRODUCTION

Since last two decades, ionic liquids (ILs) are efficiently used in organic synthesis and the most known ionic liquid is 1-butyl-3-methylimadazolium tetrafluoroborate ([bmim]BF_4). Initially, ionic liquids are introduced as an alternative green reaction medium because of their unique chemical and physical properties such as catalytic activity, high vapour pressure and non-flammable [1,2]. Since ionic liquids have proved their importance in controlling reactions as catalysts [3] and in the development of new processes under clean and environmentally benign methodologies which are sustainable for the long term. Ionic liquids are used in various organic reactions like alkylation, allylation, hydroformylation, epoxidation, Friedel-Craft reaction, Diels-Alder reaction, Knoevenagel condensation and Wittig reaction [4]. There is numerous reports are available for the application of ionic liquids to synthesize quinolines [5], but to best of our knowledge, this is the first report on utilizing ionic liquids to synthesize pyranoquinoline derivatives.

Pyranoquinoline moiety is important class of nitrogen heterocycles, which are widely employed as potential antimicrobial [6], antimalarial [7], cytotoxicity [8], anti-inflammatory [9], anticoagulant [10], anti-allergic [11], antitumor [12] and anti-parasitic activities [13]. Due to the versatile utilization of the pyranoquinoline derivatives in the field of organic synthesis as well as in pharmaceutical field, many scientists have been encouraged to develop highly efficient procedures for the preparation of pyranoquinoline derivatives with above mentioned biological activities.

Previously, different substrates like quinolones [14], pyran [15], cyclcopentane [16], nitrobenzaldehye [17] derivatives and catalysts such as $InCl_3$ [14], DABCO [18], KF-Al_2O_3 [19], piperidine [14,15], I_2/HgO [20] have been used for the synthesis of pyrano[2,3-*b*]quinoline derivatives. Based on the above insights and our continuous research on microwave irradiation in organic synthesis [21-24], herein, we report the preparation of 3-acetylpyrano[2,3-*b*]quinolin-2(1*H*)-one derivatives from 3-formylquinolin-2(1*H*)-ones and ethyl acetoacetate using [bmim]BF_4 as catalyst under microwave irradiation.

EXPERIMENTAL

Commercially available starting materials, reagents and solvents were used as such. Melting point was measured using

a Boetieus micro heating table and are uncorrected. Completion of reaction and purity of all compounds was checked by TLC plate using petroleum ether and ethyl acetate as a mobile phase. IR (KBr, cm^{-1}) spectra were obtained on Shimadzu 8201 spectrometer. NMR spectra were recorded on Bruker AMX-400 MHz spectrometer using TMS as an internal reference. Elemental analyses were performed on Perkin Elmer CHN-analyzer. High resolution Mass spectra were recorded on LCMS-Agilent 6330 Ion Trap mass spectrometer. Microwave reactions were performed in Ragas Microwave Synth System [RG3IL], complete with glass door, 700 Watt delivered power, exhaust system, triple safety interlocks, magnetic stirrer, automatic temperature control.

Synthesis of 3-acetylpyrano[2,3-*b*]quinolin-2(1*H*)-ones (3a-e): A mixture of respective 3-formylquinolin-2(1*H*)-ones (**1a-e**) (0.5 mmol), ethyl acetoacetate (**2**) (0.11 mL, 0.5 mmol) and [bmim]BF_4 was mixed thoroughly and kept in microwave reactor and irradiated for specified time at 120 ºC and power of 160 W (Table-1). Using TLC, the reaction was monitored every 30 s interval time. After the complete reaction, product was allowed to cool, poured into crushed ice and filtered. The yellowish brown colour product was obtained with good yield and purity.

3-Acetylpyrano[2,3-*b*]quinolin-2(1*H*)-one (3a): Time: 1 min, yield: 95%, m.p.: 176-178 ºC; IR (KBr, ν_{max}, cm^{-1}): 1727 (O=C=O), 1652 (C=O-CH_3); ^{1}H NMR (DMSO-d_6) δ ppm: 10.22 (s, 1H, C_4-H), 8.50 (s, 1H, C_5-H), 7.25-8.10 (m, 4H, Ar-H), 2.32 (s, 3H, $COCH_3$); ^{13}C NMR (DMSO-d_6) δ ppm: 38.87, 118.17, 122.40, 123.90, 125.22, 127.93, 128.00, 129.01, 135.68, 141.54, 142.92, 147.54, 148.00, 189.7; MS (*m/z*): 239 (M^+). Elemental analysis of $C_{14}H_9NO_3$ calcd. (found) %: C, 70.29 (70.27); H, 3.78 (3.80); N, 5.86 (5.83).

3-Acetyl-7-methylpyrano[2,3-*b*]quinolin-2(1*H*)-one (3b): Time:1 min, yield: 96%, m.p.:160-162 ºC; IR (KBr, ν_{max}, cm^{-1}): 1723 (O=C=O), 1661 (C=O-CH_3); ^{1}H NMR (DMSO-d_6) δ ppm: 10.23 (s, 1H, C_4-H), 8.41 (s, 1H, C_5-H), 7.25-7.81 (m, 3H, Ar-H), 2.41 (s, 3H, CH_3), 2.32 (s, 3H, $COCH_3$). MS (*m/z*): 253 (M^+). Elemental analysis of $C_{15}H_{11}NO_3$ calcd. (found) %: C, 71.15 (71.15); H, 4.36 (3.37); N, 5.53 (5.56).

3-Acetyl-9-methylpyrano[2,3-*b*]quinolin-2(1*H*)-one (3c): Time: 0.5 min, yield: 95%, m.p.: 135-136 ºC; IR (KBr, ν_{max}, cm^{-1}): 1679 (O=C=O), 1605 (C=O-CH_3); ^{1}H NMR (DMSO-d_6) δ ppm: 10.37 (s, 1H, C_4-H), 8.91 (s, 1H, C_5-H), 7.48-8.08 (m, 3H, Ar-H), 2.66 (s, 3H, CH_3), 2.42 (s, 3H, $COCH_3$); ^{13}C NMR (DMSO-d_6) δ ppm: 17.28, 38.80,118.07, 121.40, 122.80, 125.28, 127.99, 127.77, 129.51, 136.28, 142.55, 144.12, 148.33, 149.12, 189.5. MS (m/z): 253 (M^+). Elemental analysis of $C_{15}H_{11}NO_3$ calcd. (found) %: C, 71.15 (71.17); H, 4.36 (4.39); N, 5.53 (5.53).

3-Acetyl-7-methoxypyrano[2,3-*b*]quinolin-2(1*H*)-one (3d): Time:1 min, yield: 94%, m.p.:122-123 ºC; IR (KBr, ν_{max}, cm^{-1}): 1726 (O=C=O), 1664 (C=O-CH_3); ^{1}H NMR (DMSO-d_6) δ ppm: 10.41 (s, 1H, C_4-H), 8.90 (s, 1H, C_5-H), 7.58-8.10 (m, 3H, Ar-H), 3.66 (s, 3H, OCH_3), 2.42 (s, 3H, $COCH_3$). MS (*m/z*): 269 (M^+). Elemental analysis of $C_{15}H_{11}NO_4$ calcd. (found) %: C, 66.91 (66.93); H, 4.11 (4.13); N, 5.20 (5.22).

3-Acetyl-9-methoxypyrano[2,3-*b*]quinolin-2(1*H*)-one (3e): Time: 1 min, yield: 93%, m.p.: 164-165 ºC; IR (KBr, ν_{max}, cm^{-1}): 1699 (O=C=O), 1658 (C=O-CH_3). ^{1}H NMR (DMSO-d_6) δ ppm: 10.45 (s, 1H, C_4-H), 8.92 (s, 1H, C_5-H), 7.60-8.11 (m, 3H, Ar-H), 3.67 (s, 3H, OCH_3), 2.41 (s, 3H, $COCH_3$). MS (m/z): 269 (M^+). Elemental analysis of $C_{15}H_{11}NO_4$ calcd. (found) %: C, 66.91 (66.94); H, 4.11 (4.11); N, 5.20 (5.21).

Antimicrobial activity: All the synthesized compounds were screened for their antibacterial and antifungal activities. For preliminary screening, antimicrobial tests were conducted using the disc-diffusion method [25]. Suspension solutions of 100 µL containing 10^8 and 10^6 CFU/mL of bacteria and fungi were spread on Mueller-Hinton agar medium (MHA) and Sabouraud's dextrose agar (SDA) medium, respectively. The discs (6 mm in diameter), impregnated with 10 µL of test compounds (500 and 1000 µg/disc) at 50 and 100 mg/mL concentrations were placed on the inoculated agar. Negative controls were prepared using the same solvent (DMSO) used to dissolve test compounds. Ofloxacin (5 µg/disc) and clotrimazole (10 µg/disc) were used as positive reference standards to determine the sensitivity of each microbial species tested. The inoculated plates were incubated at 37 and 27 ºC for 24 and 72 h for bacterium and fungus strains, respectively. The antimicrobial activity was evaluated based on the zone diameter that inhibited test organisms.

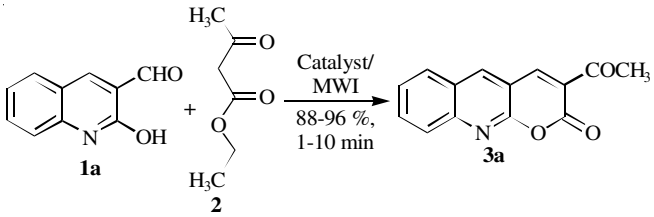
The minimum inhibitory concentration (MIC) of the synthesized compounds was estimated through the broth dilution assay for microorganisms, which were considered sensitive to compounds in the disc-diffusion assay [26]. Nutrient broth (NB) and Sabouraud's dextrose broth (SDB) were used to estimate the MIC values of the test compounds against bacteria and fungi respectively. A two fold serial dilution of test compounds were followed with 1mL of sterile broth in test tubes to provide various concentration ranges from 3.9-1000 µg/mL of the test compounds. The test organism (10 mL) was added to each tube and incubated at 37 ºC for 24 h and 27 ºC for 72 h for bacteria and fungi strains, respectively. The highest dilution of the test compound completely inhibiting the test organism was considered as MIC value of the test compound, respectively.

RESULTS AND DISCUSSION

Selection of catalyst: The selectivity of various catalysts such as DMSO, DMF, THF, TEA and [bmim]BF_4, in terms of reaction time and percentage of yield were briefly investigated. The outcome of the attempted model reaction between 3-formylquinolin-2(1*H*)-ones and ethyl acetoacetate using various catalyst (**Scheme-I**) are summarized in Table-1.

TABLE-1
EFFECT OF CATALYSTS ON SYNTHESIS OF 3-ACETYLPYRANO[2,3-*b*]QUINOLIN-2(1*H*)-ONE **3a** UNDER MICROWAVE IRRADIATION (160 W)

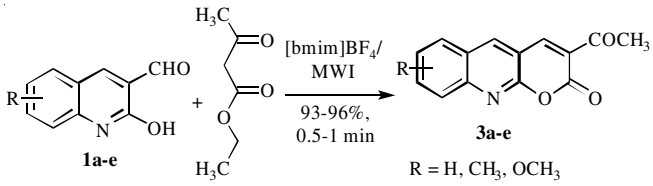
Entry	Catalyst	Reaction time (min)	Yield (%)
1	DMSO	7	92
2	DMF	8	91
3	TEA	10	89
4	THF	10	88
5	[bmim]BF_4	1	96



Scheme-I: Synthesis of 3-acetylpyrano[2,3-*b*]quinolin-2(1*H*)-one (**3a**)

From the above mentioned feasible reaction (**Scheme-I**), 3-acetylpyrano[2,3-*b*]quinolin-2(1*H*)-one was obtained with moderate to good yield. The catalysts such as DMSO, DMF, TEA and THF gave good yield but require longer reaction time (Table-1, entry 1-4). Surprisingly [bmim]BF_4 reduces the reaction time by 10 times when compared with other catalysts (Table-1, entry 5). Under optimized conditions, based on yield and reaction time, [bmim]BF_4 was proved to be the most suitable catalyst for the synthesis of 3-acetylpyrano[2,3-*b*]quinolin-2(1*H*)-one derivatives.

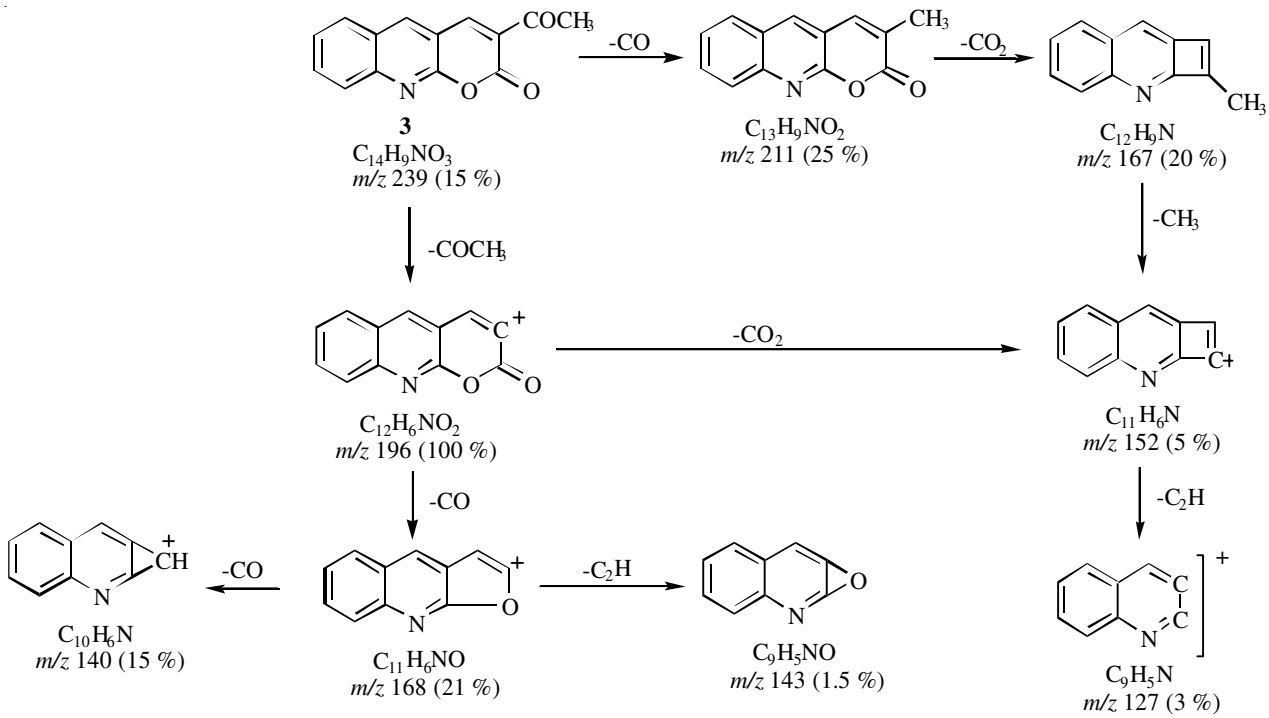
With the set of optimized reaction conditions, the reaction of substituted 3-formylquinolin-2(1*H*)-ones with ethyl acetoacetate using (bmim)BF_4 as catalyst (**Scheme-II**) was carried out. All the reactions produced corresponding pyranoquinoline derivatives in excellent yield within 1 min. The structures of newly synthesized pyranoquinoline derivatives were confirmed by IR, NMR, mass and analytical data. The IR spectrum does not show a band at 1680 cm^{-1}, which indicates the loss of aldehyde group due to condensation and showed the presence of two bands at 1727 and 1652 cm^{-1} are observed due to carbonyl groups (ring) and acetyl (-$COCH_3$), respectively. The ^{1}H NMR spectrum revealed two sharp singlets at δ 10.22 and 2.32 ppm for C_4-H and acetyl CH_3 protons, respectively, which confirmed the cyclization and formation of product **3a**. All other aromatic proton resonances were exhibited unresolved multiplet between δ 7.25-8.10 ppm and C_5-H showed singlet δ 8.50 ppm. The ^{13}C NMR spectrum show peak at δ 38.87 (CH_3), 118.17, 122.40, 123.90, 125.22, 127.93, 128.00, 129.01, 135.68, 141.54, 142.92, 147.54 (C-O), 148.00 (C=O-CH_3), 189.7 (>C=O).



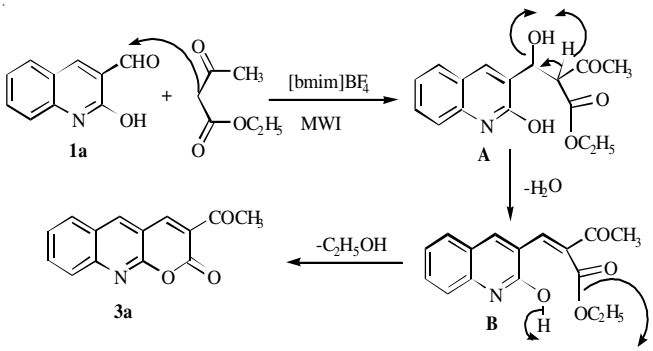
Scheme-II: Synthesis of **3a-e**

The mass spectrum indicated the molecular ion peak at m/z 239[M+] (15%). The fragmentation pattern of the mass spectrum showed a peak at *m/z* 196 (100%), which is due to the elimination of -$COCH_3$ and other fragmentations are 211 (-CO, 25%), 167 (-CO2, 20 %), 152 (-CH_3, 5 %), 127 (-C_2H, 3%), 168 (-CO, 21%), and 140 (-CO, 15%) as depicted in **Scheme-III**. Fascinatingly, the mass fragmentation of product **3a** produces a molecular ion peak at 140 (-C_2H, 15%) attributed to quinolone epoxide product, which could be a useful compound (natural product) to determine the biological functions [27].

Mechanism: The plausible mechanism for the formation of 3-acetyl-pyrano[2,3-*b*]quinolin-2(1*H*)-one is illustrated in **Scheme-IV**. In the presence of microwave irradiation, the ionic liquid [bmim]BF_4 collide with **2**, at the same time the active methylene group of **2** attack the aldehyde functional group of quinolone **1a** to form non-isolatable intermediates **A** and **B**, which further undergo cyclization to give product **3a**.



Scheme-III: Mass fragmentation of **3a**



Scheme-IV: Plausible mechanism for the formation of pyrano[2,3-*b*]quinoline **(3a)**

Antimicrobial activity: The results of the antimicrobial screening studies are given in Tables 2 and 3. The antibacterial activity data revealed that compounds **3a-e** exhibits promising activity against five microorganisms such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella aerogenes*, *Bacillus subtilis* and *Bacillus cereus*, *Aspergillus flavus*, *Lipomyces lipofera* and no activity against *Corynebacterium rubrum*. Interestingly, compounds **3c** and **3d** exhibit significant activity against most of the bacteria and fungi strains compared with standard drug such as ofloxacin and clotrimazole.

The minimum inhibitory concentration (MIC) of pyranoquinoline derivatives were estimated by broth dilution assay for the microorganisms by disc-diffusion assay. The measured

TABLE-2
in vitro ANTIMICROBIAL ACTIVITY OF **3a-e**

Microorganisms	Diameter of zone of inhibition (mm)											
	3a		**3b**		**3c**		**3d**		**3e**		**A**	**B**
	µg/disc		µg/disc		µg/disc		µg/disc		µg/disc		µg/disc	µg/disc
	500	1000	500	1000	500	1000	500	1000	500	1000	5	10
Escherichia coli (NCIM 2065)[a]	8	10	7	10	7	–	–	10	9	10	23	NT
Pseudomonas aeruginosa (NCIM 2200)[a]	7	9	–	10	7	12	7	10	–	11	22	NT
Klebsiella aerogenes (NCIM 2239)[a]	8	7	9	11	–	11	–	12	7	10	24	NT
Salmonella typhimurium (NCIM 2501)[a]	–	9	8	10	–	–	–	–	–	–	21	NT
Bacillus subtilis (NCIM 2063)[a]	9	10	9	13	10	12	10	13	7	9	24	NT
Bacillus cereus (NCIM 2155)[a]	7	11	8	10	11	12	7	9	7	10	19	NT
Vibrio fischeri (NCIM 2154)[a]	–	–	–	–	7	9	8	13	–	–	27	NT
Corynebacterium rubrum (NCIM 2252)[a]	–	–	–	–	–	–	–	–	–	–	25	NT
Staphylococcus albus (NCIM 2178)[a]	–	–	7	11	8	12	–	10	7	12	21	NT
Proteus vulgaris (NCIM 2027)[a]	–	–	8	11	–	–	–	–	–	–	19	NT
Aspergillus niger (NCIM 1196)[b]	–	–	–	–	7	11	–	10	–	–	NT	16
Aspergillus flavus (NCIM 535)[b]	7	9	8	10	7	10	9	11	8	10	NT	16
Rhodotorula rubra (NCIM 3174)[b]	–	–	–	–	11	13	10	12	8	10	NT	17
Aspergillus fumigatus (NCIM 902)[b]	8	9	–	–	–	–	–	–	–	–	NT	18
Aspergillus parasiticus (NCIM 904)[b]	–	–	–	–	7	9	7	10	–	–	NT	18
Penicillium chrysogenum (NCIM 707)[b]	8	10	7	10	7	10	9	11	–	–	NT	21
Lipomyces lipofer (NCIM 3252)[b]	7	12	9	12	8	12	8	9	8	10	NT	18
Trichoderma viridie (NCIM 1195)[b]	9	11	9	12	–	–	–	–	8	12	NT	19

[a]Bacteria, [b]Fungi ; **A** = Ofloxacin, **B** = Clotrimazole, – No inhibition, NT = Not tested.

TABLE-3
MINIMUM INHIBITORY CONCENTRATION OF **3a-e**

Microorganisms	**3a**	**3b**	**3c**	**3d**	**3e**
Escherichia coli (NCIM 2065)[a]	31.2	62.5	–	–	62.5
Pseudomonas aeruginosa (NCIM 2200)[a]	125	31.2	125	62.5	–
Klebsiella aerogenes (NCIM 2239)[a]	125	62.5	–	–	15.6
Salmonella typhimurium (NCIM 2501)[a]	–	125	62.5	–	–
Bacillus subtilis (NCIM 2063)[a]	125	125	125	62.5	125
Bacillus cereus (NCIM 2155)[a]	62.5	15.6	31.2	15.6	62.5
Vibrio fischeri (NCIM 2154)[a]	–	–	15.6	–	–
Corynebacterium rubrum (NCIM 2252)[a]	–	–	–	–	–
Staphylococcus albus (NCIM 2178)[a]	–	62.5	62.5	–	–
Proteus vulgaris (NCIM 2027)[a]	–	31.2	–	–	–
Aspergillus niger (NCIM 1196)[b]	–	–	62.5	62.5	–
Aspergillus flavus (NCIM 535)[b]	15.6	62.5	62.5	62.5	–
Rhodotorula rubra (NCIM 3174)[b]	31.2	31.2	31.2	62.5	62.5
Aspergillus fumigatus (NCIM 902)[b]	31.5	–	–	–	–
Aspergillus parasiticus (NCIM 904)[b]	–	–	–	–	–
Penicillium chrysogenum (NCIM 707)[b]	62.5	15.6	31.2	15.6	–
Lipomyces lipofer (NCIM 3252)[b]	62.5	125	62.5	62.5	31.2
Trichoderma viridie (NCIM 1195)[b]	62.5	15.6	–	–	–

MIC values of the synthesized compounds **3a-e** were found to be between 15.6 to 125 µg/mL.

Conclusion

In conclusion, a novel synthetic method for the synthesis of 3-acetylpyrano[2,3-*b*]quinolin-2(1*H*)-ones using [bmim]BF_4 as catalyst under microwave irradiation within one min is developed. This method is an efficient and eco-friendly, which offers significant preparative advantages such as operational simplicity, mild reaction conditions and very less reaction time. Synthesized pyranoquinoline derivatives shown moderate to excellent biological inhibition activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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