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Research Article

# SYNTHESIS OF 6, 7-DISUBSTITUTED-2-ARYL-4H-1-BENZOPYRAN-4-ONES AS ANTIBACTERIAL AGENTS

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**ABSTRACT**
Benzopyrones constitute one of the major classes of natural products. Both semisynthetic and natural benzopyrone derivatives are known to possess important biological properties and as a result several attempts and procedures have been reported and developed for their synthesis. The present study was designed to synthesize various substituted 2-aryl-4H-1-benzopyran-4-one derivatives. The title compounds were prepared starting from a diketone i.e. 4-substituted-2-hydroxydiaroylmethane. The structures of these newly synthesized compounds were confirmed by their analytical and spectral data. The synthesized compounds were subjected for antibacterial screening against *P.aeruginosa*, *B.subtilis* and *E.coli*. Among all the derivatives 5d showed maximum activity against *P.aeruginosa* & *E.coli* and 5b showed highest activity against *B.subtilis* & *E.coli*. The results of the antibacterial screening suggest that some of the substituted 2-aryl-4H-1-benzopyran-4-one derivatives possess appreciable antibacterial activity and may prove useful in future drug development.
**Keywords**: *P.aeruginosa*, *E.coli*, *B.subtilis*

## INTRODUCTION

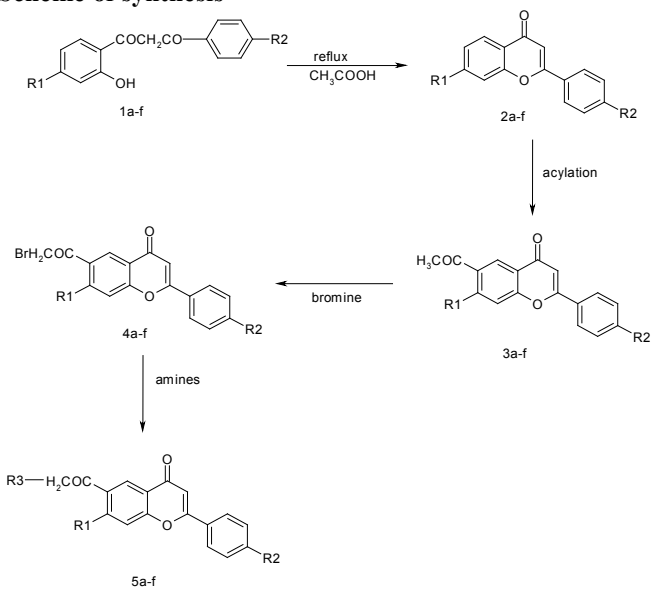
5 and 6 membered heterocycles with oxygen or nitrogen as heteroatom are gaining importance as the scientific and technical reports are constantly pouring informations to improvise the synthetic techniques involved to prepare these compounds and justifying with regard to their varied biological and pharmacological applications. Benzopyran-4-one or flavones, where a six membered oxygen containing heterocycle is fused with benzene ring, are also known as anthoxanthines. These are yellow pigments which occurred in the plant kingdom in free state or as glycoside or associated with tannins. The flavonoids are considered potential for human health as well as constitute an important part of human diet. Chemically the flavones are very closely related to anthocyanins; the flavones are hydroxylated derivatives of 2-aryl-4H-1-benzopyran-4-ones.

A retro synthetic analysis for flavones reveals 2-benzoyloxyacetophenone which can be readily formed by the benzoylation of 2-hydroxyacetophenone. Thus the derivatives of 2-hydroxyacetophenone and different benzoyl chloride can be utilized as suitable reagent equivalent for flavones. In practice the cyclisation of 2-benzoyloxyacetophenone takes place in two stages. Initially a base catalyzed rearrangement converts 2-benzoyloxyacetophenone into o-hydroxydibenzoylmethane which may be cyclised in the presence of acid to the benzopyran-4-one. Much attention has been paid to naturally occurring flavones because of their pharmacological and ecological effects. Special interest has been focussed on hydroxylated or methoxylated flavones during the last decade since they have been in the area of drug development because of their broad spectrum of pharmacological activities[1, 2]. They have been proved to have variety of biological activities such as anticarcinogenic [3-5], HIV reverse transcriptase inhibitor [6], inhibitors of oxidative reaction [7, 8], anti-inflammatory [9, 10], coronary vasodilating [11], antileukaemic [12], anticoagulant [13], CNS depressant [14] etc. Since the substituted-2-aryl-4H-1-benzopyran-4-ones derivatives are known to exhibit wide variety of pharmacological activities the available literatures prompted us to modify the benzopyrone ring to explore the activities associated with this, thus in our present study synthesis of benzopyrone derivatives condensed with various amines has been undertaken to evaluate as possible antibacterial agents.

## MATERIAL AND METHODS

The melting points were determined by open capillary tube method and are uncorrected. The IR spectra of the synthesized compounds were recorded on a Shimadzu 8400 S FTIR spectrophotometer using KBr discs in the region of 4000-400 $cm^{-1}$. NMR spectra were recorded using TMS as internal standard at 60 MHz on a Brukar Spectrospin 200 spectrometer. Mass spectral studies were carried out using JEOL GC mate instrument. Precoated Silica gel G TLC plates were used to check the purity of the compounds (benzene:ethyl acetate- 1:1 and toluene:cyclohexane- 1:1 as mobile phase), detections were done in UV chamber or by using iodine vapour.

**Scheme of synthesis**



**Synthesis of 7-hydroxy-2-(4-chlorophenyl)-4H-1-benzopyran-4-ones (2a)**

2, 4-dihydroxydibenzoylmethane (0.025mol) was dissolved in glacial acetic acid (35ml) and then added concentrated sulphuric acid (2ml). The solution was then refluxed for 2 hours with intermittent shaking. The reaction mixture was then poured on crushed ice. The product was filtered and washed with a large quantity of water repeatedly and dried. It was then recrystallized from petroleum ether (60-80$^0$C). Melting point 132$^0$C, yield 59%, $R_f$ value 0.71. Compounds 2b-f was synthesized in a similar manner. 2a IR (cm$^{-1}$): 3569 (OH str), 3067 (C-H str aromatic), 1620 (C=O str ketone), 1568 (C=C vibration C2 and C3), 1483 (C=C str aromatic), 735 (C-Cl str). 2a $^1$H NMR (δ): 6.5 1H s C-3 H, 7.2-7.4 4H m Ar H, 7.5-7.65 3H m Ar H, 11.75-11.8 1H s phenolic OH.

**Synthesis of 6-acetyl-7-hydroxy-2-(4-chlorophenyl)-4H-1-benzopyran-4-ones (3a)**

0.01 mol of 2a was added to 30 ml of nitrobenzene. Added to this 0.022 mol of freshly powdered anhydrous aluminium chloride and 0.01 mol of acetic anhydride, then it was heated for 4 hours in an oil bath and cooled. 75 gm of crushed ice was added to this followed by 4 ml of concentrated hydrochloric acid. The product obtained by filtration was dried and recrystallized from 95% ethanol. Melting point 195$^0$C, yield 58%, $R_f$ value 0.69. In a similar manner compounds 3b-f were synthesized. 3a IR (cm$^{-1}$): 3552 (O-H str), 3065 (C-H str aromatic), 2960 (C-H str aliphatic), 1618 (C=O str ketone), 1570 (C=C vibration C2 and C3), 1485 (C=C str aromatic), 740 (C-Cl str). 3a $^1$H NMR (δ): 2.7-2.8 3H s $CH_3$, 6.4 1H s C-3 H, 7.1-7.3 4H m Ar H, 7.4-7.49 2H m Ar H, 11.7-11.8 1H s phenolic OH.

**Bromination of 6-acetyl-7-hydroxy-2-(4-chlorophenyl)-4H-1-benzopyran-4-ones (4a)**

0.01 mol of 3a was added to 50 ml of glacial acetic acid. To this solution was added bromine (0.01 mol) drop wise over a period of 1 hour with constant stirring. After the addition was complete the solution was stirred till the evolution of hydrogen bromide ceases. The resulting solution was then poured onto crushed ice. Crystals were collected by filtration, dried and then recrystallized from 50% alcohol. Melting point 159$^0$C, yield 60%, $R_f$ value 0.68. Similarly compounds 4b-f were synthesized. 4a IR (cm$^{-1}$): 3560 (OH str), 3062 (C-H str aromatic), 2950 (C-H str aliphatic), 1626 (C=O str ketone), 1576 (C=C str C2 and C3), 1487 (C=C str aromatic), 742 (C-Cl str), 586 (C-Br str). 4a $^1$H NMR (δ): 3.3-3.4 2H s alkyl H, 6.5 1H s C-3 H, 7.3-7.5 4H m Ar H, 7.6-7.75 2H m Ar H, 11.72-11.81 1H s phenolic OH.

**Synthesis of 6-acetylamino-7-hydroxy-2-(4-chlorophenyl)-4H-1-benzopyran-4-ones (5a)**

The amine (pyrrolidine and dimethylamine) (0.01 mol) was added to a solution of bromo derivative (4a-f, 0.01 mol) in absolute alcohol (25 ml) and the mixture was heated under reflux for about 2 hours on water bath. A crystalline solid was separated out on standing overnight in cold condition. Filtered, washed with cold water and dried. The compounds 5b-l were synthesized in a similar manner. Physical data are reported in table 1. 5a IR (cm$^{-1}$): 3539 (OH str), 3059 (C-H str aromatic), 2948 (C-H str aliphatic), 1640 (C=O str ketone), 1568 (C=C str C2 and C3), 1495 (C=C str aromatic), 1200 (C-N str amine), 748 (C-Cl str). 5a $^1$H NMR (δ): 2.7-2.81 2H alkyl H, 3.0-3.3 6H aliphatic H, 6.6 1H s C-3 H, 7.32-7.54 4H m Ar H, 7.68-7.79 2H m Ar H, 11.7-11.8 1H s phenolic OH.

**Table 1. Physical data of synthesized derivatives**

| Comp code | R1 | R2 | R3 | Mol. Formula | Mol. Wt | Yield (%) | M.P ($^0$C) | $R_f$ value |
|---|---|---|---|---|---|---|---|---|
| 5a | -OH | -Cl |  | $C_{19}H_{16}O_4NCl$ | 357.5 | 61 | 175 | 0.69 |
| 5b | -OH | -Cl |  | $C_{19}H_{16}O_4NCl$ | 383.5 | 70 | 168 | 0.72 |
| 5c | -OH | -Br |  | $C_{19}H_{16}O_4NBr$ | 402 | 63 | 177 | 0.68 |
| 5d | -OH | -Br |  | $C_{21}H_{18}O_4NBr$ | 428 | 68 | 179 | 0.67 |
| 5e | -OH | -$OCH_3$ |  | $C_{20}H_{19}O_5N$ | 353 | 75 | 161 | 0.73 |
| 5f | -OH | -$OCH_3$ |  | $C_{22}H_{21}O_5N$ | 379 | 69 | 170 | 0.66 |
| 5g | -$CH_3$ | -Cl |  | $C_{20}H_{18}O_3NCl$ | 355.5 | 67 | 165 | 0.64 |
| 5h | -$CH_3$ | -Cl |  | $C_{22}H_{20}O_3NCl$ | 381.5 | 72 | 182 | 0.72 |
| 5i | -$CH_3$ | -Br |  | $C_{20}H_{18}O_3NBr$ | 400 | 65 | 167 | 0.71 |
| 5j | -$CH_3$ | -Br |  | $C_{22}H_{20}O_3NBr$ | 426 | 71 | 185 | 0.74 |
| 5k | -$CH_3$ | -$NO_2$ |  | $C_{20}H_{18}O_5N_2$ | 366 | 64 | 201 | 0.62 |
| 5l | -$CH_3$ | -$NO_2$ |  | $C_{22}H_{20}O_5N_2$ | 392 | 63 | 189 | 0.66 |

**Antibacterial activity**

The title compounds (5a-l) were subjected for antibacterial screening in their DMF solution by cylinder plate method against *P.aeruginosa*, *B.subtilis* and *E.coli*. Nutrient agar while hot was poured into sterilized petridishes and allowed to attain room temperature. The agar plates were inoculated with test culture by spreading uniformly with sterile swabs. 1mg of the test compound was dissolved in 1ml of DMF and 0.1 ml of this solution was used for testing the antibacterial activity. The zone of inhibition after an incubation for 24 hours at $37^0$C measured in millimetre and represented by (-), (+), (++) and (+++) depending upon the diameter of the inhibited zone in microbial growth. The control ($CHCl_3$) showed no activity under identical conditions. Norfloxacin which was used as standard showed a zone of inhibition of 28mm against *P.aeruginosa* & *B.subtilis* and 24.5mm against *E.coli*

**RESULTS AND DISCUSSION**

The title compounds were synthesized from the convenient starting material 2-hydroxy-4-substituted diaroylmethane (1a-f). The structure of compounds 2a-f were confirmed by their NMR spectra, where it showed signals for one proton ranging from δ 6.4-6.6 which was identified as the C3 proton of the benzopyrone ring and thus confirming cyclisation of the diketone (1a-f) to give 2a-f. The NMR spectra of 3a-f showed three alkyl protons ranging from δ 2.7-2.8 as a sharp intense singlet. Downfield shift of signals for these protons in compound 4a-f took place after the bromination on the α-carbon of the acyl group, giving a α-haloketone, where it shows signals for two methylene protons at δ 3.3-3.4. Continued further the title compounds (5a-l) were synthesized by reacting the brominated derivatives with two amines i.e piperidine and dimethylamine. The six alkyl protons of 5a were appeared rather downfield at δ 3.0-3.3 due to the deshilding effect of electronegative nitrogen. The synthesized compounds shown their characteristic absorption band in IR spectra in the region of 3539-3569 $cm^{-1}$ for OH stretching, 3059-3067 $cm^{-1}$ for CH stretching aromatic, 1618-1840 $cm^{-1}$ C=O stretching ketone, 1487-1570 $cm^{-1}$ C=C stretching aromatic.

**Table- 2. Antibacterial activity of the synthesized compounds**

| Compound code | Zone of inhibition (in mm) | | |
|---|---|---|---|
| | *P. aeruginosa* | *B. subtilis* | *E. coli* |
| 5a | + | ++ | + |
| 5b | ++ | +++ | +++ |
| 5c | + | ++ | ++ |
| 5d | +++ | ++ | +++ |
| 5e | ++ | + | + |
| 5f | +++ | ++ | ++ |
| 5g | ++ | ++ | + |
| 5h | ++ | + | ++ |
| 5i | - | + | - |
| 5j | ++ | + | + |
| 5k | - | + | + |
| 5l | ++ | + | + |

- = inactive, + = weakly active (12-15 mm), ++ = moderately active (16-19 mm), +++ = highly active (20-25 mm)

It is interesting to note from the observations of antibacterial screening that some of the compounds are effective against all the three strains of bacterial species although with a degree of variation. The results for antibacterial studies revealed that compound 5d & 5f exhibited highest activity against *P.aeruginosa*, compound 5b was highly active against *B.subtilis* while compound 5b & 5d were most effective against *E.coli*. whereas the compounds 5a, 5c, 5e, 5g, 5h, 5j & 5l showed weak to moderate activity and the rest of the compounds were found to be almost inactive.

**CONCLUSION**

As per the results of the present study, it could be concluded that the substituted 2-aryl-4H-1-benzopyran-4-ones conjugated with two different amines showed good antibacterial activity against *P.aeruginosa*, *B.subtilis* and *E.coli*. Thus in future there is very scope to explore the 2-aryl-4H-1-benzopyran-4-one moiety further to develop antibacterial agents more effectively.

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