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DESIGN AND SYNTHESIS OF SOME γ-BENZOPYRONE DERIVATIVES FOR ANTIBACTERIAL ACTIVITY

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ABSTRACT

Antimicrobial agents are widely used in the management of infectious disease but as an inevitable fact, most of them have consequently developed resistance to the micro-organisms. In the present study some γ-benzopyrone derivative were synthesized and reported. The prepared analogues were subjected to physicochemical studies like melting point determination, TLC and percentage yield. The structures of all title compounds were characterized by IR, Mass and NMR spectroscopy. Antibacterial screening of newly synthesized compounds 3a-i was carried out against *Staphylococcus aureus, Pseudomonus aeruginosa, Bacillus subtilis and Escherichia coli*. Compounds 3a, 3b, 3c, 3d and 3e were appreciably active against the organisms whereas compound 3f was active moderately.

INTRODUCTION

There is a growing interest in the pharmacological potential of natural products. Oxygen containing heterocycles have a great potential to become a clinically useful drug. γ-benzopyrone is classified as a member of the benzopyrone family of compounds, all of which consist of a benzene ring annulated with a pyrone ring. Flavonoids are among the most ubiquitous groups of plant secondary metabolites distributed in various foods and medicinal plants. The synthesis of benzopyrones and their derivatives has attracted considerable attentions from organic and medicinal chemists for many years as a large number of natural products contain this heterocyclic nucleus.

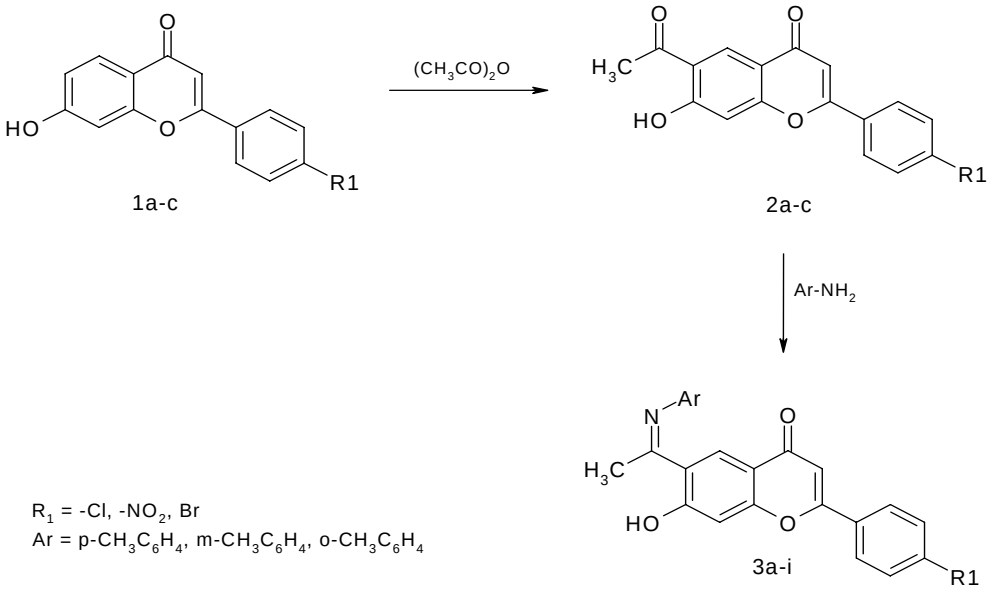
The benzopyrones can be subdivided into the α-benzopyrones to which the coumarins belong and the γ-benzopyrones, of which the flavonoids are significant members. Benzopyrones and its derivatives can be synthesized by various methods, which include Pechmann reaction, Perkin reaction, Knovenegal reactions etc. The plant extracts containing this particular heterocycles, which were employed as herbal remedies in ancient years, have now been extensively studied for their diverse biological activities. Benzopyrones are used in the pharmaceutical industry as a precursor in the synthesis of a number of synthetic anticoagulant dicoumarol, warfarin and some even more potent rodenticides. The literature survey revealed that benzopyrones with phenolic hydroxyl groups are capable of scavenging reactive oxygen species which may be responsible for their anti-inflammatory activity. These are widely used as additives in food, perfumes, cosmetics, pharmaceuticals and optical brighteners and would dispersed fluorescent and laser dyes. Recently 4-methyl-7-hydroxy coumarin has been reported to have anti skin-cancer activity. In vivo studies have also revealed the role of coumarins in hepatotoxicity. A lot of attentions have been drawn on the study of flavonoids as inhibitors of oxidative reactions[1-3] and as anti-inflammatory agents[4-6]. It was found that enhanced activities of coumarins are dependent on its nucleus. The recognition of key structural features within benzopyrone family is crucial for the design and development of new analogues with improved activity and for the establishment of their mechanism of action and potential side effects. These are extremely variable in structure due to the various types of substitutions in their basic structure, which can influence their biological activity. The 4-methylcoumarin derivatives present in various naturally occurring compounds, are known to exhibit a wide range of biological and pharmaceutical activities. Compounds having Chromone moiety are associated with interesting physiological activities such as

antibacterial, antiviral, anticancer, antioxidant, antifungal, anticholesteremic, antidiabetic, antiallergic, diuretic etc[7]. Propolis, collected by honey bees from different parts of plants which is also a resinous hive product, is a rich source of flavonoids[8]. Significant amounts of the isoflavone genistein as its glucosyl glucoside has also been reported in the tubers of the American groundnut[9]. There are numerous scientific and technical reports available regarding in vitro studies designed to exploit the pharmacological and biological properties of benzopyrone class of compounds, however these studies may not be sufficient enough or yet to easily realize the mode of action in vivo. In view of the above observations it was our interest in this present investigation to synthesize and evaluate some γ-benzopyrone analogues for antibacterial activity.

MATERIALS AND METHODS

All the chemicals used for current investigation were of analytical grade. The melting points were determined by open capillary tube method and are uncorrected. The progress of reaction and the purity of the compounds were checked by using precoated Silica gel G plates; (mobile phase- benzene: ethyl acetate- 1:1); detections were carried out either in UV chamber or by using iodine vapour. The IR spectra were recorded using KBr discs in the region of 4000-400 cm^{-1} in a Shimadzu 8400S FTIR spectrophotometer. ^{1}HNMR spectra were recorded using TMS as internal standard on a Brukar Spectrospin 200 spectrometer. Mass spectral analysis was carried out by using JEOL GC mate instrument.

Scheme:



Acylation of 7-hydroxy-2-(4-chlorophenyl) benzopyran-4-one (2a)

To 50 ml of nitrobenzene γ-benzopyrone (0.01mol, 1a) was added. To this mixture 0.01mol of acetic anhydride and freshly powdered 0.022 mol of anhydrous aluminium chloride were also added and then it was heated in an oil bath for around 5 hours. The flask was then removed, kept aside for some times and cooled. To this 100gm of crushed ice was added followed by 5ml of concentrated hydrochloric acid. The product obtained was filtered and recrystallised from 95% ethanol. Similarly compounds 2b-c were synthesized.

General procedure for the synthesis of title derivatives (3a-i)

A solution of the suitable amine (0.01 mol) in 50ml of alcohol was taken in a flask and to this solution was added 2a (0.01 mol) in 35ml of alcohol. An organic base, piperidine (5-6 drops) was also added. This reaction mixture was then heated under reflux for around 5 hours. Then it was cooled to room temperature and poured into 150 ml of water containing ice. The product was filtered and washed with cold water, dried and recrystallized from aqueous ethanol. In a similar manner compounds 3b-i were synthesized. The physical data are reported in table 1.

Antibacterial evaluation:

The title compounds were screened for antibacterial activity by cup plate Agar diffusion method. The microorganisms used were *Staphylococcus aureus, Pseudomonus aeruginosa, Bacillus subtilis and Escherichia coli*. Agar plates were prepared by using melted agar which when hot was poured onto the petridishes and allowed to solidify. Then the plates were inoculated over the surface of the media with sterile cotton. The bores were then made and filled with the solution of suitable concentration of the test compounds and standard and then incubated at 37^0C for 24 hours. The antimicrobial agents slowly diffused around the cups and produced a particular zone of inhibition of the microbial growth which was then calculated (Table 2). The control ($CHCl_3$) with solvent (DMF) under identical conditions did not show any activity. Ciprofloxacin was used as a standard.

RESULTS AND DISCUSSION

Some γ-benzopyrone derivatives condensed with different amines were synthesized and evaluated by a 3 step linear synthesis. All the chemicals used were of analytical grade. The title compounds were synthesized in a moderate to good yields. The thin layer chromatographic studies were performed using Silica gel G plates; mobile phase used for development of the chromatogram was benzene: ethyl acetate- 1:1; detections of spots were

carried out either in UV chamber or by using iodine vapour. The title compounds were characterized by their analytical and spectral data. The spectral analysis performed depending upon the IR, ^{1}HNMR and Mass spectral information. The IR spectra of the synthesized compounds exhibited characteristic absorption bands for stretching and bending vibrations for O-H, C-H aromatic, C-H aliphatic, C=O, C=N, C=C aromatic, C-H aliphatic etc. A careful observation of the NMR spectrum of compound 3a revealed the presence of two singlet peaks at δ2.08 and 2.68 which was analyzed and assigned as the signals for two methyl groups present; one on the aromatic framework, another on the imino carbon atom. Both these two alkyl groups were deshielded to somewhat greater extent, because one of them was attached with the aromatic ring and another one was joined to a carbon which had the double bond. Thus these electronegative effects of aromatic π cloud as well as the doubly bonded π electrons deshielded those alkyl protons and appeared at a downfield value. The one proton attached to the C-3 was resonated at δ 5.24 for compound 3a. Again for the same reason i.e. due to the presence of π electrons between C-2 and C-3 this proton was deshielded too. Further it was also noticed that the aromatic protons of 2nd side chain, the 6th imino side chain and the benzopyrone ring itself, were appeared as a multiplet in the aromatic region. The mass spectral data showed the M+1 ion peak i.e. the molecular ion peak from which the molecular weights of all the synthesized compounds were confirmed. The antibacterial evaluation revealed important informations that the compounds like 3a, 3b, 3c, 3d and 3e were active against the bacterial strains appreciably, whereas compound 3f was active moderately. Remaining compounds were either slightly active or inactive.

3a-IR (KBr, cm^{-1}): 3585 (O-H str), 3067 C-H str aromatic, 2945 C-H str aliphatic, 1739 C=O str, 1671 C=N str, 1448 C=C str aromatic, 1352 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.08 3H CH_3, 2.68 3H CH_3, 5.24 1H C-3 H, 6.63 8H Ar H, 6.95 2H Ar H, 10.65 1H OH. Mass (M+H): 404.95

3b-IR (KBr, cm^{-1}): 3560 (O-H str), 3057 C-H str aromatic, 2932 C-H str aliphatic, 1735 C=O str, 1664 C=N str, 1458 C=C str aromatic, 1362 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.04 3H CH_3, 2.72 3H CH_3, 5.31 1H C-3 H, 6.65 8H Ar H, 6.87 2H Ar H, 10.69 1H OH. Mass (M+H): 404.93

3c-IR (KBr, cm^{-1}): 3580 (O-H str), 3072 C-H str aromatic, 2944 C-H str aliphatic, 1742 C=O str, 1663 C=N str, 1457 C=C str aromatic, 1342 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.05 3H CH_3, 2.61 3H CH_3, 5.31 1H C-3 H, 6.69 8H Ar H, 6.91 2H Ar H, 10.60 1H OH. Mass (M+H): 404.97

3d-IR (KBr, cm^{-1}): 3573 (O-H str), 3059 C-H str aromatic, 2940 C-H str aliphatic, 1735 C=O str, 1654 C=N str, 1461 C=C str aromatic, 1348 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.03 3H CH_3, 2.71 3H CH_3, 5.35 1H C-3 H, 6.67 8H Ar H, 6.97 2H Ar H, 10.64 1H OH. Mass (M+H): 415.48

3e-IR (KBr, cm^{-1}): 3548 (O-H str), 3061 C-H str aromatic, 2948 C-H str aliphatic, 1739 C=O str, 1664 C=N str, 1451 C=C str aromatic, 1358 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.13 3H CH_3, 2.78 3H CH_3, 5.41 1H C-3 H, 6.62 8H Ar H, 6.98 2H Ar H, 10.69 1H OH. Mass (M+H): 415.45

3f-IR (KBr, cm^{-1}): 3560 (O-H str), 3047 C-H str aromatic, 2940 C-H str aliphatic, 1746 C=O str, 1672 C=N str, 1449 C=C str aromatic, 1364 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.19 3H CH_3, 2.69 3H CH_3, 5.38 1H C-3 H, 6.61 8H Ar H, 6.94 2H Ar H, 10.65 1H OH. Mass (M+H): 415.47

3g-IR (KBr, cm^{-1}): 3559 (O-H str), 3057 C-H str aromatic, 2945 C-H str aliphatic, 1740 C=O str, 1679 C=N str, 1459 C=C str aromatic, 1371 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.09 3H CH_3, 2.67 3H CH_3, 5.34 1H C-3 H, 6.62 8H Ar H, 6.91 2H Ar H, 10.67 1H OH. Mass (M+H): 449.38

3h-IR (KBr, cm^{-1}): 3563 (O-H str), 3057 C-H str aromatic, 2958 C-H str aliphatic, 1751 C=O str, 1663 C=N str, 1448 C=C str aromatic, 1378 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.11 3H CH_3, 2.63 3H CH_3, 5.37 1H C-3 H, 6.59 8H Ar H, 6.88 2H Ar H, 10.71 1H OH. Mass (M+H): 449.41

3i-IR (KBr, cm^{-1}): 3565 (O-H str), 3050 C-H str aromatic, 2964 C-H str aliphatic, 1758 C=O str, 1669 C=N str, 1468 C=C str aromatic, 1388 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.12 3H CH_3, 2.67 3H CH_3, 5.41 1H C-3 H, 6.53 8H Ar H, 6.79 2H Ar H, 10.75 1H OH. Mass (M+H): 449.37

CONCLUSION

In the present investigation some γ-benzopyrone derivatives were synthesized by a two step process and reported thereafter the antibacterial activity. Out of all the synthesized compounds 3a, 3b, 3c, 3d and 3e were active enough against the bacteria, whereas compound 3f was active moderately. As far as the antibacterial activity is concerned these derivatives can be designed, modified and synthesized for their better yield and efficacy.

Table- 1. Data of the synthesized derivatives:

Compound code	Mol. Formula	Mol. Wt	Melting point (^{0}C)	R_f value	Yield (%)
3a	$C_{24}H_{18}ClNO_3$	403.87	249	0.62	59
3b	$C_{24}H_{18}ClNO_3$	403.87	252	0.59	47
3c	$C_{24}H_{18}ClNO_3$	403.87	241	0.54	52
3d	$C_{24}H_{18}N_2O_5$	414.42	251	0.58	51
3e	$C_{24}H_{18}N_2O_5$	414.42	266	0.61	53
3f	$C_{24}H_{18}N_2O_5$	414.42	255	0.56	57
3g	$C_{24}H_{18}BrNO_3$	448.32	269	0.63	51
3h	$C_{24}H_{18}BrNO_3$	448.32	251	0.53	44
3i	$C_{24}H_{18}BrNO_3$	448.32	267	0.61	55

Table- 2. Antibacterial activity of the derivatives.

Compound code	Zone of inhibition			
	S. aureus	*P. aeruginosa*	*B. subtilis*	*E. coli*
3a	++	+	++	+
3b	+	++	+	+
3c	++	+	++	+
3d	+	++	+	+
3e	+	++	++	+
3f	++	+	+	+
3g	-	+	+	-
3h	+	-	-	+
3i	-	+	-	+

- = inactive, +++ = highly active (18-22 mm), ++ = moderately active (13-17 mm), + = weakly active (8-12 mm)

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