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SCHIFF BASES OF γ-BENZOPYRONE: SYNTHESIS, CHARATERIZATION AND BIOLOGICAL ACTIVITY

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ABSTRACT

The present investigation deals with the synthesis, characterization and evaluation of antibacterial activity of some γ-benzopyrones. The derivatives were synthesized in a moderate to good yields. The structural confirmation was based on the IR, NMR and Mass spectral studies. The anti bacterial activity of the compounds was evaluated against *Staphylococcus aureus, Pseudomonus aeruginosa, Bacillus subtilis and Escherichia coli*. The zone of inhibitions was measured as the parameter of activity. Out of all derivatives 3a, 3b, 3c, 3d and 3e were appreciably effective against the bacterial strains used for the study. Other compounds were either slightly effective or ineffective. These analogues can be further modified for better efficiency.

INTRODUCTION

Benzopyrone derivatives have attracted considerable attention by the scientists, as they are widely used as fragrances, pharmaceuticals and agrochemicals and are often reported in literature because of their diverse pharmacological as well as biological properties. Their wide range of activities made these compounds attractive for investigators for further modification and screening as novel drug candidates. Benzopyrone comprises a group of naturally occurring compounds found in a variety of plant sources. The synthesis and pharmacological activity of these analogues has become an important aspect in medicinal chemistry because of their wide variety of applications. Benzopyrone family of compounds, all of which consist of a benzene ring fused to a pyrone ring, can be subdivided into the benzo-α-pyrones to which the coumarins belong and the benzo-γ-pyrones of which the flavonoids are the principal members**.** There are four main sub-types of coumarins: simple coumarins, furano coumarins, pyrano coumarin and the pyrone-substituted coumarins. Coumarin is the parent substance of the benzo-α-pyrone group which was first isolated from in 1820. It is the simplest compound of a large class of naturally occurring phenolic substances. Benzopyrones are naturally occurring as well as synthetic derivatives, and having widespread applications as HIV protease inhibitors, anticoagulant, spasmolytic and bacteriostatic agents. The prototypical compound is known as 1,2-benzopyrone or as *o*-hydroxycinnamic acid and lactone, and it has been well studied. However, the most widely reported activities for benzopyrone derivatives are their anti-inflammatory and anti-cancer activities[1-5]. Although distributed throughout all parts of the plant, the benzopyrones, in general, occur at the highest levels in the fruits, seeds, roots, leaves, latex, green tea and other foods such as chicory. They are also found at high levels in some essential oils such as cassia oil, cinnamon bark oil etc. Environmental conditions and climatic changes could influence the occurrence and concentration of coumarins in different plant parts.

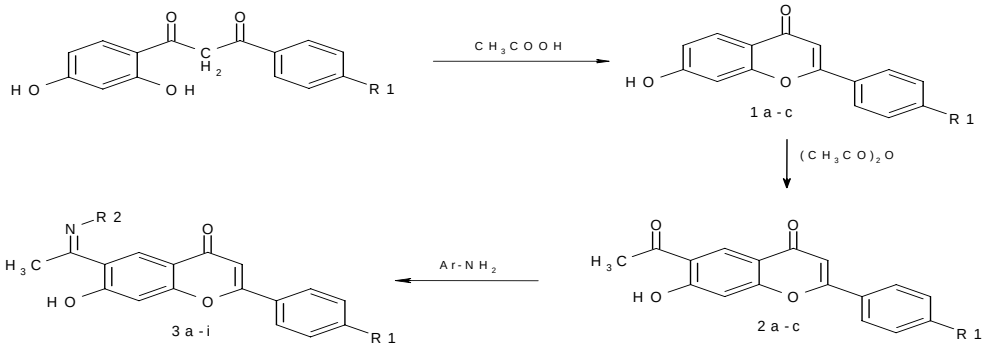
The simple benzopyrones are the hydroxylated, alkylated or alkoxylated derivatives of the parent compound coumarin along with their glycosides. Furano-coumarins and pyrano-coumarins consist of a five-membered furan ring and a six-membered pyran ring attached to the coumarin nucleus respectively. Flavonoids are among the most ubiquitous groups of plant secondary metabolites distributed in various foods and medicinal plants. During the past decades an increasing number of reports are available on the beneficial effects of flavonoids[6-14]. Although they are sometimes found as their aglycones, like flavone, flavonol, flavanone,

and flavanol, flavonoids most commonly occur in plant materials as flavonoid O-glycosides, in which one or more hydroxyl groups of the aglycones are bound to a sugar. There are some hydroxyl groups such as 7-hydroxyl group in flavones, flavanones, and isoflavones and the 3- and 7- hydroxyl groups in flavonols and flavanols in flavonoids that are usually glycosylated. Quercetin is one of the most abundant flavonol-type flavonoids found in fruits and vegetables, such as apples and onions, and is a strong antioxidant. The prominent flavonoids in tea are the flavanols catechin, epicatechin, epicatechin gallate, epigallocatechin and epigallocatechin gallate. There are almost about 900 isoflavone aglycones occurring naturally which can be divided into major classes on the basis of difference in carbon structures[15]. These polyphenols show biological properties through their free radical- scavenging antioxidant activities, for their ability to inhibit enzymes such as protein kinase[16-18]. Based on the above observations and considering their biological importance in this present study here it is reported the synthesis and biological activity of Schiff bases of some γ-benzopyrone.

MATERIALS AND METHOD

The research chemicals used in this investigation were of analytical grade. The melting points of the synthesized compounds were determined by open capillary tube method and are uncorrected. The IR spectra were recorded in a Shimadzu 8400S Fourier Transform Infra Red spectrophotometer using KBr discs in the region of 4000-400 cm^{-1}. The ^{1}HNMR spectra were recorded on a Brukar Spectrospin 200 spectrometer using TMS as internal standard and the values are expressed in δ scale (ppm). Mass spectra were obtained by using Micro Mass Q-P instrument (Waters). Precoated Silica gel G plates were used to check the purity of the compounds; Benzene: ethyl acetate- 1:1 used as developing solvent; detections were carried out either in UV chamber or by using iodine vapour.

Scheme of synthesis:



Synthesis of 7-hydroxy-2-(4-chlorophenyl)-benzopyran-4-one (1a)

0.05 mol of 2, 4-dihydroxybenzoyl-4-chlorobenzoyl methane was taken in 80ml of glacial acetic acid and 5ml of concentrated sulphuric acid was then added with shaking. The solution was then refluxed for about 3 hours with intermittent shaking. The reaction mixture was then treated with crushed ice. After allowing settling down, the product was filtered and washed repeatedly with cold water and air dried. It was recrystallised from petroleum ether (60-80^{0}C). Melting point 136^{0}C, yield 68%, R_f value 0.62. In a similar manner compounds 1b-c were synthesized.

Synthesis of 6-acetyl-7-hydroxy-2-(4-chlorophenyl)-benzopyran-4-one (2a)

7-hydroxy-2-(4-chlorophenyl)-benzopyran-4-one (1a) (0.01mol) was added to 50 ml of nitrobenzene. Acetic anhydride (0.01mol) and freshly powdered anhydrous aluminium chloride (0.022mol) were added and then the reaction mixture was heated at 150^{0}C for around 5 hours or till the evolution of gas ceases. The reaction content was then cooled and to this added 100 gm of crushed ice and 4 ml of concentrated HCl. The product was filtered and recrystallised from 95% ethanol. Melting point found 184^{0}C, yield 53%, R_f value 0.65. Similarly compounds 2b-c was synthesized.

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

A solution of the appropriate amine (0.01 mol) in 50ml of alcohol was added to 2a (0.01 mol) in 35ml of alcohol. To this mixture piperidine (5-6 drops) was also added and then heated under reflux for 5 hours. The reaction mixture was then allowed to cool to room temperature and poured into 150 ml of water containing ice. The product was filtered and washed with cold water and dried. Recrystallization was done by aqueous ethanol. In a similar manner compounds 3b-i were synthesized. The physical data are reported in table 1.

Antibacterial Evaluation

All the synthesized title compounds were screened for antibacterial activity by cup plate Agar diffusion method. The bacterial strains used were *Staphylococcus aureus, Pseudomonus aeruginosa, Bacillus subtilis and Escherichia coli*. Agar plates were prepared by using agar, melted and then poured onto the petridishes and allowed to solidify. Then the plates were inoculated with sterile cotton over the surface of the media. The bores were filled with the solution of sample and standard then incubated at 37^{0}C for 24 hours. Antimicrobial agents slowly spread around cups and produced a characteristic zone of inhibition of the microbial growth which was then calculated (Table 2). Under identical condition the control ($CHCl_3$) with solvent (DMF) did not show any activity. Ciprofloxacin was used as a standard.

RESULTS AND DISCUSSION

Different Schiff bases of γ-benzopyrone were synthesized from 2, 4-dihydroxybenzoyl-4-substitutedbenzoyl methane. The cyclisation of these diketone to get benzopyrone moiety was undertaken by treating with acetic acid and concentrated sulphuric acid, where concentrated sulphuric acid acts as the cyclizing agent. The resulting γ-benzopyrone compound was then acetylated by using acetic anhydride and anhydrous aluminium chloride to introduce an acetyl side chain on the aromatic ring which resulted in the formation of 6-acetyl derivatives of γ-benzopyrone. These 6-acetyl derivatives of γ-benzopyrone were then subjected for reaction with different amines resulting in the formation of Schiff bases. The compounds synthesized were then assigned and characterized by the following methods: Melting point determination; Thin layer chromatography- precoated silica gel G plates were used to determine the R_f values of the synthesized compounds and benzene: ethyl acetate- 1:1 was used as mobile phase. After the development of chromatogram the spots were detected either by iodine vapour or in UV chamber; IR spectroscopy- the IR spectra of the synthesized compounds were recorded in the region of 4000-400 cm^1 using KBr discs on a Shimadzu 8400S Fourier Transform IR spectrophotometer; NMR spectroscopy- the proton NMR spectral analyses were carried out in a Bruker Spectrospin-200 instrument; Mass spectrometry- Mass spectra of the synthesized compounds were recorded using a Micro Mass Q-P instrument (Waters) using Elctrospray Ionization. The IR spectra of final synthesized compounds exhibited characteristic absorption bands for O-H stretching, C-H stretching aromatic, C-H stretching aliphatic, C=O stretching, C=N stretching, C=C stretching aromatic, C-H bending aliphatic etc. The ^{1}HNMR spectrum of 1a shown the proton resonance at δ 2.18 for three hydrogens which was confirmed as the methyl protons attached to 6^{th} position imino side chain. The C-3 proton was resonated at around δ 5.14 for compounds 3a. This proton was deshielded more by the presence of π electrons associated with the C-2 and C-3. The aromatic protons of 2^{nd} phenyl, the benzopyrone ring itself and the aromatic ring at imino side chain was appeared at their respective aromatic regions. The hydroxyl proton being phenolic was appeared at δ 10.65 for compound 3a. The mass spectra of the final compounds showed characteristic M+1 peaks from which their molecular weights were confirmed. The antibacterial study revealed that compounds 3a, 3b, 3c, 3d and 3e were effective appreciably against the organisms. Other compounds were either slightly effective or ineffective.

3a-IR (KBr, cm^{-1}): 3572 (O-H str), 3053 C-H str aromatic, 2938 C-H str aliphatic, 1730 C=O str, 1677 C=N str, 1441 C=C str aromatic, 1347 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.18 3H CH_3, 5.14 1H C-3 H, 6.63 8H Ar H, 6.91 2H Ar H, 10.65 1H OH. Mass (M+H): 435.89

3b-IR (KBr, cm^{-1}): 3569 (O-H str), 3064 C-H str aromatic, 2948 C-H str aliphatic, 1739 C=O str, 1670 C=N str, 1452 C=C str aromatic, 1337 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.14 3H CH_3, 5.11 1H C-3 H, 6.61 8H Ar H, 6.95 2H Ar H, 10.62 1H OH. Mass (M+H): 435.83

3c-IR (KBr, cm^{-1}): 3578 (O-H str), 3059 C-H str aromatic, 2931 C-H str aliphatic, 1735 C=O str, 1666 C=N str, 1447 C=C str aromatic, 1351 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.17 3H CH_3, 5.15 1H C-3 H, 6.58 8H Ar H, 6.89 2H Ar H, 10.59 1H OH. Mass (M+H): 435.85

3d-IR (KBr, cm^{-1}): 3575 (O-H str), 3071 C-H str aromatic, 2926 C-H str aliphatic, 1735 C=O str, 1662 C=N str, 1445 C=C str aromatic, 1341 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.21 3H CH_3, 5.14 1H C-3 H, 6.67 8H Ar H, 6.94 2H Ar H, 10.69 1H OH. Mass (M+H): 446.45

3e-IR (KBr, cm^{-1}): 3563 (O-H str), 3059 C-H str aromatic, 2940 C-H str aliphatic, 1745 C=O str, 1673 C=N str, 1439 C=C str aromatic, 1345 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.18 3H CH_3, 5.13 1H C-3 H, 6.61 8H Ar H, 6.97 2H Ar H, 10.62 1H OH. Mass (M+H): 446.42

3f-IR (KBr, cm^{-1}): 3571 (O-H str), 3053 C-H str aromatic, 2948 C-H str aliphatic, 1755 C=O str, 1679 C=N str, 1442 C=C str aromatic, 1352 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.16 3H CH_3, 5.17 1H C-3 H, 6.65 8H Ar H, 6.94 2H Ar H, 10.67 1H OH. Mass (M+H): 446.48

3g-IR (KBr, cm^{-1}): 3575 (O-H str), 3059 C-H str aromatic, 2954 C-H str aliphatic, 1735 C=O str, 1664 C=N str, 1447 C=C str aromatic, 1363 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.17 3H CH_3, 5.15 1H C-3 H, 6.62 8H Ar H, 6.90 2H Ar H, 10.65 1H OH. Mass (M+H): 480.38

3h-IR (KBr, cm^{-1}): 3564 (O-H str), 3062 C-H str aromatic, 2950 C-H str aliphatic, 1739 C=O str, 1665 C=N str, 1437 C=C str aromatic, 1358 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.13 3H CH_3, 5.12 1H C-3 H, 6.67 8H Ar H, 6.93 2H Ar H, 10.62 1H OH. Mass (M+H): 480.32

3i-IR (KBr, cm^{-1}): 3565 (O-H str), 3071 C-H str aromatic, 2959 C-H str aliphatic, 1737 C=O str, 1679 C=N str, 1451 C=C str aromatic, 1362 C-H bending aliphatic. ^{1}HNMR ($CDCl_3$, δ ppm): 2.15 3H CH_3, 5.14 1H C-3 H, 6.62 8H Ar H, 6.97 2H Ar H, 10.64 1H OH. Mass (M+H): 480.34

CONCLUSION

The approach of the present study was to synthesize various Schiff bases of γ-benzopyrone and evaluate the anti bacterial activity. In the light of antibacterial evaluation it can be stated that the Schiff bases of γ-benzopyrone can lead to a promising tool to be modified and studied further for improving their efficacy.

Table- 1. Data of the synthesized derivatives:

Compound code	Mol. Formula	Mol. Wt	Melting point (^{0}C)	R_f value	Yield (%)
3a	$C_{23}H_{15}ClN_2O_5$	434.84	267	0.62	51
3b	$C_{23}H_{15}ClN_2O_5$	434.84	249	0.59	54
3c	$C_{23}H_{15}ClN_2O_5$	434.84	257	0.57	42
3d	$C_{23}H_{15}N_3O_7$	445.39	264	0.58	56
3e	$C_{23}H_{15}N_3O_7$	445.39	268	0.63	57
3f	$C_{23}H_{15}N_3O_7$	445.39	259	0.67	54
3g	$C_{23}H_{15}BrN_2O_5$	479.29	261	0.64	58
3h	$C_{23}H_{15}BrN_2O_5$	479.29	266	0.58	49
3i	$C_{23}H_{15}BrN_2O_5$	479.29	278	0.59	48

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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