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RESEARCH ARTICLE

Evaluation of *In Vitro* Anti-Inflammatory Activity for Novel Imine Derivatives from Acetylcoumarin by Catalyst-Free Green Solvent

P. N. BALAJI[1*], K. C. LAVANYA[1] and D. RANGANAYAKULU[2]

[1]Department of Pharmaceutical Chemistry, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati. A.P, India

[2]Department of Pharmacology, Sri Padmavathi School of Pharmacy, Tiruchanoor, Tirupati. A.P, India

balajipn47@gmail.com

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Abstract: Facile method used in synthesis of some novel seven Schiff's base (azomethine) compounds in catalytic free green solvent (*i.e* using glycerol) at ambient temperature from acetylcoumarin. Acetoxy coumarin are synthesized by acetylating from previously prepared 4-methyl-7-hydroxy coumarin with acetic anhydride from standard procedures, that was further heated in metallic catalyst to produce 8-acetly-7-hydroxy-4-methylcoumarin by fries re-arrangement. The obtain acetylcoumarin was reacted with different substituted arylamine in glycerol under catalytic free condition heated at 90 ^{o}C for 6 h to produce imine derived coumarin compounds. Compounds were recrystalised and its melting points were determined by digital apparatus which are un-correct. Structure of the compounds was characterized by FT-IR (KBr method) and ^{1}H NMR for selected compounds. All the synthesized compounds were evaluated for *in-vitro* anti-inflammatory by standard procedure with slight revision. From the obtain data compounds shows substantial result compared to standard value.

Keywords: Acetylcoumarin, Green solvent, Azomethine, Fries rearrangement, Ambient temperature

Introduction

In the field of science and technology an advance method of techniques are employed in field of drug design and development. The most commonly used organic chemicals or in catalytic organic liquid medium causes worst impact on our environment, to avoid this in search of selection of non-hazardous green solvents become the great challenge in field of organic preparation[1]. Glycerol plays an important key role as green solvent in various organic preparations because of its wide role as it is atoxic, bio-degradable and re-cyclable liquid and is chemically inert, high stable with high boiling range and also disperses maximum organic compounds that are poorly miscible in water[2-4]. These advantages make it ideal for use as a sustainable solvent in organic synthesis. Coumarin a bicyclic ring characterized under benzo-alfa-pyrones which are abundantly found in nature. The structure of coumarin posses a wide pharmacological application which mainly found in anti- coagulant

(warfarin), treating high protein edema in combination with troxerutin, immunomodlater in chronic infection, anti-cancer, antimalarial, antimicrobial, antibacterial, antifungal, anti-neurodegenerative, anti-oxidative, antiviral, antiparasitic, anti-inflammatory analgesic, anti-diabetic, anti-depressive so on[5-7]. Schiff's base are also called imine derivatives which are formed by nucleophilic attack of aryl amine on electrophilic group of aldehyde or ketone in liberation of water called azomethine[8-10]. This imine containing heterocyclic compounds posse's a wide biological and pharmacological applications. In this concern an attempt is made to synthesize some novel imine derivatives from coumarin and evaluated for *in vitro* anti-inflammatory by protein denaturation inhibition.

Experimental

Chemicals used for the above mentioned work was purchased from Himedia, Merck, Thermo-Fischer syntifics, SRL. Melting point for the synthesized imine derivatives were determined by Siska thermo digital melting point apparatus without further correction, TLC for the prepared compounds were performed by using pre-coated commercial available silica impregnated paper, spots were detected by iodine chamber. All the prepared compounds were characterized (Table 1) and identified by FT-IR by KBr method using analytical technologies (FT-IR) spectrophotometer 2202. Selected compounds were subjected to ^{1}H NMR spectra recorded on Bruker 300 MHz in DMSO[11]. All the compounds were screened for *in vitro* anti-inflammatory activity and the results are shown in the Table 2.

Table 1. Physical and characteristic data

Comp	Molecular formula, (Wt)	Melting Point, ^oC	% Yield	FT-IR (KBr) cm^{-1}	^{1}H NMR δ value (DMSO)ppm	R_f value
4a	$C_{18}H_{15}NO_3$ (293.3)	202	72	C-C(1568),C=C(1605),C=O(1710), C-O-C(1265),C=N(1688),C-N(1340), C-OH(3440)	7.2 -8.8 (m, 8H, Ar-H), 11.3(s, 1H,OH), 2.4 (s,3H,CH_3)	0.68
4b	$C_{18}H_{16}N_2O_3$ (308.3)	216	67	C-C(1550),C=C(1615),C=O(1708), C-O-C(1260),C=N(1670),C-N(1340), C-OH(3447)	-	0.70
4c	$C_{19}H_{15}NO_5$ (337.3)	262	55	C-C(1530),C=C(1620),C=O(1730), C-O-C(1265),C=N(1690),C-N(1340), C-OH(3435)	-	0.78
4d	$C_{18}H_{14}N_2O_5$ (338.3)	220	68	C-C(1560),C=C(1605),C=O(1714), C-O-C(1265),C=N(1688),C-N(1335), C-OH(3444)	7.1 -8.6 (m, 7H, Ar-H), 11.4(s, 1H, OH), 2.6 (s,3H,CH_3)	0.74
4e	$C_{18}H_{14}NO_3F$ (311.4)	278	70	C-C(1568),C=C(1605),C=O(1710), C-O-C(1265),C=N(1688),C-N(1340), C-OH(3435)	7.4 -8.9 (m, 7H, Ar-H), 11.3(s, 1H,OH), 2.2 (s,3H,CH_3)	0.62
4f	$C_{18}H_{15}NO_4$ (309.3)	244	80	C-C(1568),C=C(1605),C=O(1715), C-O-C(1265),C=N(1688),C-N(1340), C-OH(3433)	-	0.68
4g	$C_{19}H_{17}NO_4$ (323.3)	232	78	C-C(1526),C=C(1610),C=O(1710), C-O-C(1260),C=N(1680),C-N(1343), C-OH(3440)	7.3 -8.4 (m, 7H, Ar-H), 11.2(s,1H, OH), 3.44 (s, 3H, OCH_3), 2.3(s, 3H, CH_3)	0.80

Chloroform: Ethyl acetate [8:2]

Table 2. *In vitro* anti-inflammatory activity

S.No	Sample	% Inhibition of denaturation
1.	Control	-
2.	Ibuprofen	84.33
3.	**4a**	66.15
4.	**4b**	52.45
5.	**4c**	59.35
6.	**4d**	63.70
7.	**4e**	51.56
8.	**4f**	69.75
9.	**4g**	65.59

*Synthesis of 7-acetoxy-4-methylcoumarin from 7-hydroxy-4-methylcoumarin (**1,2**)*

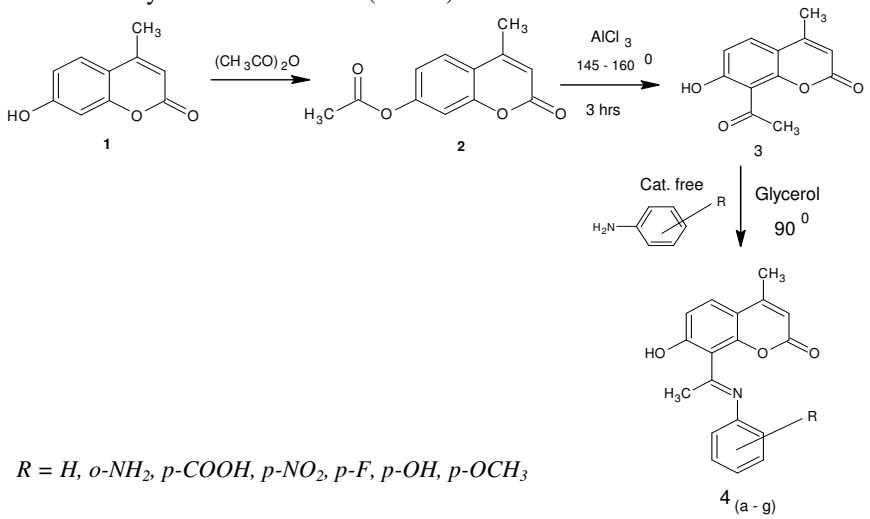
It was prepared as per the literature method[12] (Scheme 1).

Conversion of compound (2) to 8-acetyl-7-hydroxy-4-methylcoumarin (3)
(Fries rearrangement mechanism)

7-Acetoxy-4-methylcoumarin (0.1 M) prepared (**2**) from the literature method was mixed with anhydrous $AlCl_3$ (0.5 mol) in 250 mL R.B flask and heated under anhydrous conditions in an oil bath at 120 ^{o}C for 30 min and the temperature was raised to 145-160 ^{o}C and maintained for 2-3 h. And later crushed ice was added to this mixture and acidified with 1 N HCl in drop wise with stirring the mixture and left for 2-3 h in order to decompose the complex. The separated product was filtered (**3**), washed with water and recrystalised from ethanol. M.P:186 ±2 ^{o}C.

*Synthesis of imine derivatives of acetylcoumarin (**4a-g**)*

An equal concentration 0.01 M of (**3**) was mixed in 10 mL of glycerol and added in small quantity of aryl amine to it in a 100 mL Rb flask. The mixture was condensed on water bath at temperature of 90 ^{o}C (optimum to get good yield) without catalyst. Condensation was continued for 5-6 h (reaction time based on TLC). The reaction mixture was cooled and quenched with cold water to precipitate out. The precipitate was filtered and washed with cold water and recrystalised in benzene (20 mL) to obtain desired Schiff base.



R = H, o-NH_2, p-COOH, p-NO_2, p-F, p-OH, p-OCH_3

Scheme 1. Synthesis of imine derivatives of acetylcoumarin

In vitro screening

Anti-inflammatory by protein denaturation inhibition

The imine derived coumarin compounds were subjected to screen for *in-vitro* anti-inflammatory activity to determine its potency in inhibition of bovine albumin denaturation on thermal induction technique, procedure used with slight modification[13-15].

The stock solution of test compounds were prepared by adding 0.1% w/v (*i.e*.1000 µg/mL) in a few mL of DMSO and finally diluted to 10 mL with tris buffer and pH 6.8 was maintained using glacial acetic acid. Meanwhile standard ibuprofen also prepared in the same manner. Test solution and standard solution of 1 mL (*i.e* 100 µg/mL) concentration of synthesized compounds and drug (IBF) was mixed with 1 mL of 0.2% w/v bovine albumin solution prepared in tris buffer (adjusted to pH 6.8 using glacial acetic acid) taken in test tube covered with aluminum foil and incubated it at 37 ^{0}C in BOD incubator for 15 min, and later denaturation was induced by keeping the reaction mixture at ~70 ^{0}C on heating water bath for 5 min. After cooling, the turbidity was measured at 660 nm (UV-Visible Spectrophotometer (Analytica). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average was taken. Results are illustrated in Table 2.

Results and Discussion

The compounds prepared in the study of novel imine derivatives *i.e* 7-hydroxy-4-methyl-8-[(1*E*)-*N*-phenylethanimidoyl]-2*H*-chromen-2-one and its derivatives were characterized by FT-IR by KBr method shows peak absorbance of functional group at C=O at 1710 cm^{-1}, C=N at 1680 cm^{-1}, C-O-C at 1250 cm^{-1} respectively, and ^{1}H-NMR in DMSO by TMS internal standard shows 7.2 – 8.6 ppm Aromatic protons, 11.2 ppm coumarin OH this makes confirmation of the structure of the compounds, activity to check the potency of the compounds on inhibition of protein denaturation by thermal induction method. The compound **4f >4a>4g>4d** shows prominent anti-inflammatory property and rest are good to moderate.

Conclusion

The synthesized novel imine compounds from acetylcoumarin by fries rearrangement process shows structural confirmation by IR and NMR. A prominent anti-inflammatory activity was achieved by this, later these compounds are used to evaluate various biological activities and also make some structural rearrangement for improving its nature.

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