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Design, Synthesis, Antibacterial and *invitro* Antioxidant activity of substituted 2*H*-Benzopyran-2-one derivatives

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Abstract: A series of some coumarinyl and chromen sulfanyl derivatives have been synthesized by conventional methods and characterized on the basis of IR and NMR spectral data. The title compounds were subjected for antibacterial activity against both gram positive and gram negative organisms and *invitro* antioxidant activity by 1,1-Diphenyl-2,2-picryl hydrazyl free radical (DPPH) method.
Keywords : Coumarin, Antibacterial, Antioxidant.

Introduction

Coumarins (2*H*-1-benzopyran-2-ones) are important oxygen containing fused heterocycles used in drugs and dyes. The incorporation of other heterocyclic moiety either as substituent group or as a fused component into parent coumarin alters the property of parent coumarin and converts it into a more useful product[1]. Coumarins are plant flavonoids widely distributed in nature. Natural coumarins are known to have anabolic, antioxidant and hepatoprotective activities[2]. Substituted coumarin derivatives have been reported to have variety of biological activities viz., anticoagulant (coumadin, sodium warfarin), HIV protease inhibition[3], CNS depressant, hypnotic, sedative, diuretic, analgesic and antitubercular activities[4].

The potent antibiotics like Novobiocin, Coumaromycin and Chartesium are coumarin derivatives. Recently, the interest on these compounds has been revived owing to their use as fluorescent markers in the biochemical determination of enzymes. Coumarin derivatives are also known for their dyeing properties and are available as laser dyes and optical brightners[5]. Coumarin derivatives can be synthesized by one of such methods as the Claisen rearrangement[6], Perkin reaction[7], Pechmann reaction[8], Witting reaction[9] as well as the Knoevenagel condensation[10].

Thiazoles are one of the most intensively investigated class of aromatic five membered heterocycles. Thiazole derivatives find a new variety of applications ranging from bacteriostatics, antibiotics, CNS and high ceiling diureties[11]. All these facts were driving force to develop novel thiazole derivatives with wide structural variation. Thus thiazole derivatives play pivotal role in medicinal chemistry. Phthalimide derivatives constitute an important class of compounds possessing diverse

biological properties including antimicrobial, antimalarial, antihypertensive and antiviral activities[12].

Materials and Methods

Experimental

Melting point of the compounds synthesized was determined by using Thiel's melting point apparatus and are uncorrected. The IR spectra of the synthesized compounds were recorded using KBr pellet method in the range of 4000-500 cm^{-1} on a Fourier Transform IR Spectrophotometer, Shimadzu 8700 and frequencies were recorded in wave numbers. ^1H NMR-(400 MHz) spectra was recorded on AMX-400 MHz spectrometer using $CDCl_3$. Chemical shifts (δ) are reported in parts per million (ppm) down field from internal reference TMS. Purity of the compounds were checked by thin layer chromatography using silica gel-G coated aluminium plates as stationary phase, *n*-hexane : ethyl acetate as mobile phase.

Procedure

Synthesis of 3-acetyl-2*H*-chromen-2-one

Salicylaldehyde and ethyl acetoacetate were stirred and cooled. Piperidine was added with shaking and the mixture was maintained at freezing temperature to get a yellow solid mass which was recrystallized from ethanol to get the target compound.

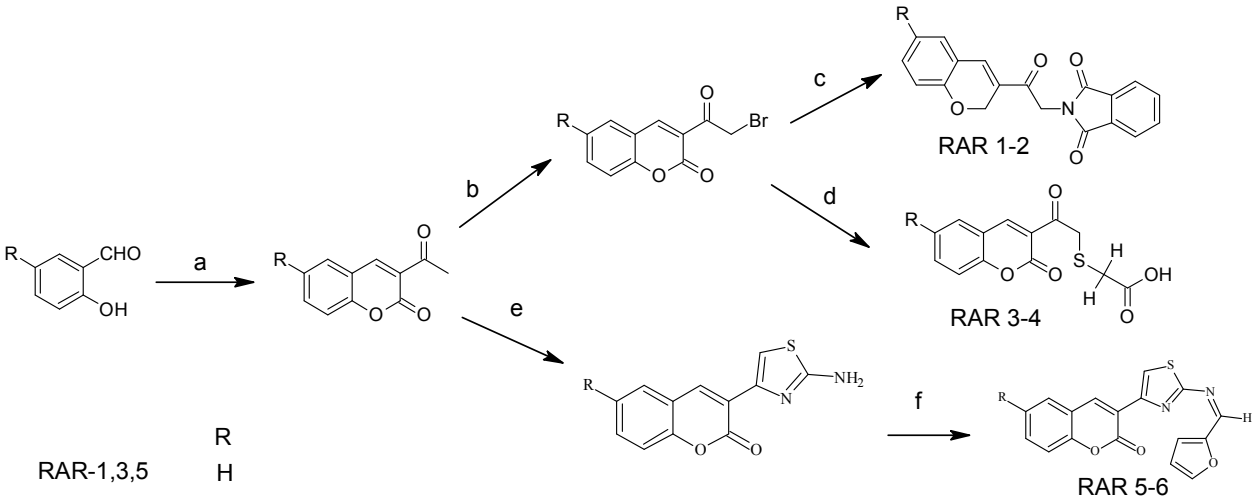
Synthesis of 3-(bromoacetyl)-2*H*-chromen-2-one

To a solution of 3-acetyl-2H-chromen-2-one in alcohol free chloroform, bromine in chloroform was added. The mixture was heated for 15 mins, cooled and filtered to get a solid mass which on washing with diethyl ether gave the desired product which was recrystallized from acetic acid.

Synthesis of 2-[2-(2*H*-chromen-3-yl)-2-oxoethyl]-1*H*-isoindole-1,3(2*H*)-dione (RAR-1)

3-(bromoacetyl)-2*H*-chromen-2-one was refluxed with 1*H*-isoindole-1,3(2*H*)-dione in the presence of dry pyridine for 8 hr and was poured into crushed ice containing 2-4 drops of concentrated hydrochloric acid. The product obtained was filtered, dried and recrystallized. All other compounds (**RAR 2-RAR 6**) were synthesized by following the same procedure. The physical data of the synthesized compounds are given in **Table 1.**

Scheme of synthesis:



	R
RAR-1,3,5	H
RAR-2,4,6	Br

a)ethylaceto acetate b)Br $_2$/$CHCl_3$ C) phthalimidine d)thioacetic acid e)thiourea/Iodine f)furan-2-carboxaldehyde

RAR-1: IR vmax(cm^{-1}): 3062 (Ar C-H str), 1728 (C=O str of pyrone), 1658 (C=O), 1598 (Ar C=C str), 1019 (C-O str).
RAR-2: IR vmax(cm^{-1}): 3082 (Ar C-H str), 1726 (C=O str of pyrone), 1666 (C=O str), 1030 (C-O str), 1589 (Ar C=C str), ^{1}HNMR (δ, ppm): 7.27-7.80 (m, Ar-H, 7H; s, CH, 1H), 4.96 (s, CH_2, 2H).
RAR-3: IR vmax(cm^{-1}): 3401 (O-H str), 2905 (Ar C-H str), 1726 (C=O str of pyrone),1679 (C=O str),1207 (C-O str), 1600 (Ar C=C str)
RAR-4: IR vmax(cm^{-1}): 3400 (O-H str), 3074 (Ar C-H str), 1699 (C=O str of pyrone), 1267 (C-O str), 1596 (Ar C=C str), ^{1}HNMR (δ, ppm): 7.22-8.01 (m, Ar-H, 3H; s, CH, 1H), 9.796 (s, O-H, 1H), 4.12 (s, CH_2, 2H), 3.44 (s, CH_2, 2H).
RAR-5: IR vmax(cm^{-1}): 3082 (Ar C-H str), 1714 (C=O str of pyrone), 1604 (C=O str), 1106 (C-O str),1600 (Ar C=C str), ^{1}HNMR (δ, ppm): 7.44-8.50 (m, Ar-H, 8H; s, CH, 1H), 7.26 (s, C-H,1H).
RAR-6: IR vmax(cm^{-1}): 3072 (Ar C-H str), 1724 (C=O str of pyrone), 1706 (C=O str), 1064 (C-O str), 1598 (Ar C=C str)

Physical property evaluation

As two of the designed compounds of the present study showed good antibacterial and antioxidant activity when compared with the standard, a computational study for prediction of ADME properties of the molecules was performed by determination of lipophilicity, TPSA and simple molecular descriptors used by Lipinski in formulating his "rule of five" calculations by using ACD lab software, ChemDraw Ultra and www.molinspiration.com. Table 1 represents the calculated ClogP, SMV, TPSA and other Lipinski parameters of the synthesized compounds RAR 1-6**.** Polar surface area and lipophilicity favors for a molecule to cross the biological membranes. Very high TPSA value contributes for a low bioavailability for the molecule. The study of molecular properties of any small molecule can be considered as a unique tool in the field of drug design and also proves that there is a relationship between the physical parameter and the biological activity. Each structure was fully geometry optimized using the ChemDraw Ultra version 8.0 force field.

Biological Activity:

Antibacterial Activity

All the synthesized compounds were tested for their antibacterial activity against both gram positive and gram negative organisms viz., *Bacillus subtilis* (NCIM 2697)*, Staphylococcus auereus* (NCIM 2079)*, Escherichia coli* (NCIM 2065) *and Klebsella pneumonia* (NCIM 5082). The activity was performed by following the procedure of cup plate agar diffusion method[14]. A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the microorganisms. 0.1 mL of inoculums (of 10^4 to 10^6 CFU / mL population prepared from standardized culture, adjusted with peptone water) was spread on the agar plate by spread plate technique. Accurately measured (0.1 mL) solution of each sample and standard were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 ºC for a period of two hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 ºC for 24 h. The presence of definite zones of inhibition around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of DMSO, which was used as a solvent for sample. The results are shown in Table II.

Table 1: Physical Data of the Synthesized Compounds

Sl NO	Comp Code	Mol Formula	Mol Wt	M.P (°C)	% Yield	R_f	ClogP[a]	TPSA[b]	HBD[c]	HBA[d]	nrotb[e]	Ref[f]
Lipinski[h]			≤500				≤5.0	≤5	≤10			
1	RAR-1	$C_{19}H_{13}NO_4$	333	184	83.0	0.40	2.12	86.358	0	6	3	87.946
2	RAR-2	$C_{19}H_{10}BrNO_5$	412	198	67.4	0.74	2.99	86.358	0	6	3	95.569
3	RAR-3	$C_{13}H_{10}O_5S$	278	135	87.0	0.31	0.73	84.581	1	5	5	70.002
4	RAR-4	$C_{13}H_9BrO_5S$	357	123	78.6	0.83	1.60	84.581	1	5	5	77.625
5	RAR-5	$C_{17}H_{10}N_2O_3S$	322	295	67.0	0.47	2.55	68.607	0	5	3	88.031
6	RAR-6	$C_{17}H_9BrN_2O_3S$	401	281	82.0	0.54	3.42	68.607	0	5	3	95.653

a= ClogP value, b= molar volume (A^3), c= topological polar surface area, d=hydrogen bond donor, e= hydrogen bond acceptor, f= number of rotatable bonds, g= refractivity (A^3), h= Lipinski's Rule of 5 for pharmaceuticals [13]

Table II: Antibacterial and Antioxidant activity of the synthesized compounds

Sl.No	Comp. Code	Antioxidant Activity	Zone of Inhibition in mm			
		% inhibition	*B.subtilis* (NCIM 2697)	*S.aureus* (NCIM 2079)	*E.coli* (NCIM2065)	*K.pneumonia* (NCIM 5082)
1	RAR 1	61	21	22	20	--
2	RAR 2	58	24	24	22	--
3	RAR 3	56	20	21	20	--
4	RAR 4	56	22	24	26	10
5	RAR 5	54	24	20	24	--
6	RAR 6	58	25	24	26	10
	Ciprofloxacin		27	26	28	29
	Ascorbic acid	81				

Antioxidant Activity

Free radical scavenging activity of the test compounds were determined by the 1,1- diphenyl picryl hydrazyl (DPPH) assay method[15]. Drug stock solution (1 mg mL–1) was diluted to final concentrations of 2, 4, 6, 8 and 10 mg mL–1 in methanol. DPPH methanol solution (1 mL, 0.3 mmol) was added to 2.5 mL of drug solutions of different concentrations and allowed to react at room temperature. After 30 min the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity. Methanol was used as the solvent and ascorbic acid as the standard. The standard drug used was ascorbic acid at a concentration of 10 µg/ml. The results in percentage of inhibition is shown in Table II.

Results and Discussion

All the synthesized compounds have been characterized by IR and ^{1}H NMR spectral data and evaluated for their antibacterial and *in-vitro* antioxidant activity. Some of the synthesized compounds exhibited good antibacterial and antioxidant activity. The compounds with bromo substitution on the heteroaryl group has shown better antibacterial activity while the compound with phthalimidine substitution has shown significant antioxidant activity and those with sulfanyl groups have exhibited moderate activity.

This research work reveals that the presence of a thiol and a carboxylic group as a substitution would lead to enhanced binding of the molecule to the bacterial enzyme and inhibit their growth. Hence the molecules **RAR-2** and **RAR-6** can be taken up for further research studies.

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