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Synthesis of Novel Flavone Acyl Esters and Correlation of log P Value with Antioxidant and Antimicrobial Activity

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Three series of flavones namely 6-hydroxy flavone, 6-chloro-3-hydroxy flavone, 3,6-dihydroxy flavone were synthesized. They were further benzoylated with different aromatic acid chlorides in the presence of pyridine gave three of acyl esters series (SCF 1 to 8, SHF 1 to 8 and SDF 1 to 8). The yields of all the substituted flavones were found satisfactory. These compounds were purified, characterized by their spectral data. log P and pKa value. They were screened for *in vitro* radical ($DPPH^{\cdot}$) scavenging activity, showed appreciable activity. 7 compounds showed antimicrobial activity against Gram-positive bacteria. Further, the MIC values of these 7 compounds were also determined. All the compounds showing antioxidant activity also showed antibacterial activity. The relationship between log P, antioxidant and antibacterial activity was established.

Key Words: Flavone acyl esters, Antioxidant, Antibacterial.

INTRODUCTION

There is growing interest in the pharmacological potential of natural products such as, flavonids, coumarins, alkaloids, glycosides, terpenoids *etc.* Flavonoid class of compounds occurs in nature as flavones, isoflavones, neoflavones *etc.* Flavonoids having chromone (γ-benzopyrone) moiety are associated with interesting physiological activities such as antibacterial, antiviral, anticancer, antioxidant, antifungal, hypolipidemic, antidiabetic, antiallergic, diuretic etc[1]. Flavonoids, the derivatives of chromones are polyhydroxylated and they are capable of selectively reacting with free radicals or systems related to the induction of inflammatory processes.

Quercetin (3,3',4',5,7-pentahydroxy flavone) and related flavonoids are known to inhibit the growth of tumor cells and to potentiate cytotoxicity of DNA damaging anticancer drugs such as *cis*-platin[2]. Very few data are available on the influence of

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lipophilic substituents on the antioxidant or antiinflammatory activity of this class of natural products[3]. Flavones inhibit CYP1A-mediated 7-ethoxyresorufin *o*-deethylase activity in rat and human liver microsomes[4]. Certain bromoflavones are found to significantly induce quinone reductase activity, which is an important mechanism of chemoprevention[5]. Since, flavonoids are known to have wide variety of pharmacological activities, many available literatures prompted us to modify benzopyrone ring to explore newer activities associated with this nucleus. Thus, in this study, attempts were made to synthesize substituted flavones and study the influence of substituents on the lipophilicity, dissociation constant and evaluate their antioxidant and antibacterial property both qualitatively and quantitatively.

EXPERIMENTAL

The chemicals used were AR grade and LR grade, purchased from Loba Chemicals, Qualigens, NR Chemicals, Rolex, Lancaster, Sigma, Reachem, S.D. Fine Chemicals, Merck and Hi-Media.

Detection method: Melting points of the test compounds synthesised were determined using Thiele's melting point apparatus) and were found uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC) using silica gel G as stationary phase and combination of benzene:chloroform; benzene:ethyl acetate and benzene:ethanol as mobile phase. The spots resolved were visualized using iodine chamber. pH of the synthesized compounds were recorded using pH meter to determine pKa values. The UV-visible spectra was recorded in the range of 200 to 800 nm on UV-visible spectrophotometer (Model Shimadzu 1601). Absorbance was taken at the λ_{max} characteristic for each test compound and the wavelengths are recorded in nm.

The IR spectra were recorded on a Fourier Transform IR Spectrometer (Model Shimadzu 8700) using KBr disc method in range of 4000-500 cm^{-1}. ^1H NMR (400 MHz) spectra were recorded on AMX-400 liquid state NMR spectrometer (Indian Institute of Science, Bangalore) in $CDCl_3$ using tetramethylsilane as an internal standard. Mass spectra was recorded on Sciex-3000 (Applied BioSystems, Canada) LC-MS-MS, 'Triple Quadruple MS' using Electro spray Ionization Positive ion mode (Indian Institute of Science, Bangalore).

General procedure for synthesis of compounds

Preparation of 2,5-dihydroxy acetophenone: Finely powdered mixture of dry hydroquinone diacetate (0.2 M) and anhydrous aluminium chloride (0.65 M) were taken in a 500 mL round bottom flask immersed in an oil bath, fitted with an air condenser protected by a $CaCl_2$ guard tube, heated at 110-120 °C for 0.5 h. When the evolution of hydrogen gas begins, the temperature was raised to 160-165 °C and maintained for 3 h. The reaction mixture was cooled and treated with 280 g of crushed ice followed by 20 mL concentrated HCl to decompose excess of $AlCl_3$. The resulting solid was filtered and washed with two 80 mL portion of cold water. The crude product was recrystallized from rectified spirit (yield 60 %).

Preparation of 2,5-dibenzoyloxy acetophenone: To a mixture of 2,5-dihydroxy acetophenone (0.2 mol) and benzoyl chloride (0.28 mol) in a flask, 60 mL of dry, redistilled pyridine was added. The contents of the flask were shaken well and were poured into a beaker containing 800 mL of 1 M HCl containing 400 g of crushed ice. The solid separated out was filtered and washed with ice-cold methanol and then with water. The crude product was recrystallized from methanol (yield 60 %).

Preparation of 3-phenyl 5-chloro-2-hydroxy acrylophenone: 100 mL of 10 % NaOH and 60 mL of rectified spirit were taken in a beaker immersed in an ice bath, (0.2 mol) 2-hydroxy-5-chloro acetophenone was added and stirred until the mixture was evenly mixed. To this (0.2 mol) benzaldehyde was added with stirring for 2 h at 25 °C. The reaction mixture was left overnight in refrigerator, filtered the solid, washed with ice-cold water until the washings were neutral to litmus. Further, the product was washed with cold rectified spirit, dried and recrystallized using rectified spirit (yield 80 %).

Preparation of 6-chloro-3-hydroxy-2-phenyl-4-benzopyrone (P-1): 3-Phenyl-5-chloro-2-hydroxyacrylophenone (0.13 mol) was taken in 325 mL methanol, 65 mL of 20 % aqueous NaOH solution was added, cooled to 0 °C. 65 mL of 30 % H_2O_2 was added to the moisture at temperature below 10 °C, stirred for 2 h and poured on to crushed ice, neutralized by dilute HCl solution. The solid separated out (**P-1**) was filtered, washed with ice-cold water, dried and recrystallized from rectified spirit (yield 76 %).

Preparation of 2,5-dihydroxy dibenzoyl methane: 2,5-Dibenzoyloxy acetophenone (0.15 mol) was dissolved in 135 mL of dry pyridine in a flask and was heated to 50 °C. Further, 7.3 g of finely powdered KOH was added with stirring to get the solid precipitate. The reaction mixture was cooled to room temperature, acidified with 10 % acetic acid solution, filtered, dried and recrystallized from rectified spirit (yield 67 %).

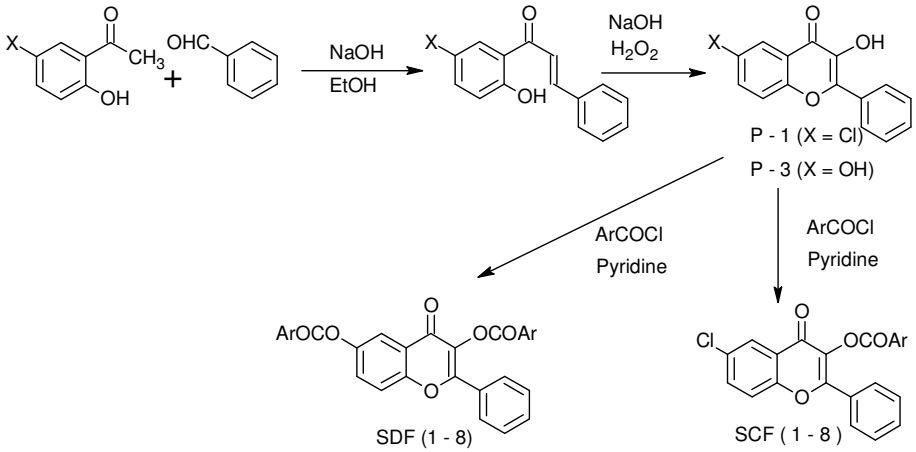
Preparation of 6-hydroxy-2-phenyl-4-benzopyrone (P-2): 2,5-Dihydroxy dibenzoyl methane (0.1 mol) was dissolved in 75 mL of glacial acetic acid and 4 mL of concentrated H_2SO_4 was added with constant stirring, refluxed for 2 h and cooled. The product was precipitated in crushed ice, filtered and recrystallized from *n*-hexane (yield 72 %).

Preparation of 3-phenyl-2,5-dihydroxyacrylophenone: 2,5-Dihydroxy acetophenone (0.2 mol) was added to 100 mL of 10 % ethanolic NaOH in a beaker immersed in an ice bath. To this mixture, benzaldehyde (0.2 mol) was added with stirring for 2 h at 25 °C. Reaction mixture was left overnight in refrigerator and the solid separated was filtered, washed with ice cold water until the washings were neutral to litmus. The product was then washed with cold rectified spirit, dried and recrystallized from rectified spirit (yield 74 %).

Preparation of 3,6-dihydroxy 2-phenyl-4-benzopyrone; (P-3): To 3-phenyl-2,5-dihydroxy acrylophenone (0.13 M) in 325 mL methanol, 65 mL of 20 % aqueous NaOH was added and the mixture was cooled to 0 °C. 65 mL of 30 % H_2O_2 was

added to the mixture at below 10 °C, stirred for 2 h and then poured into crushed ice, neutralized with dilute HCl. The solid so obtained was filtered, washed with ice-cold water, dried and recrystallized from *n*-hexane (yield 67 %).

Preparation of 6-chloro-2-phenyl esters of γ-benzopyrones (SCF series, Scheme-I): To (0.01 mol) 6-chloro-2-phenyl-3-hydroxy-4-benzopyrone (**P-2**) and (0.014 mol) acyl acid chlorides, 2.5 mL of dry redistilled pyridine was added, shaken well and poured into 10 mL of 1 M HCl containing 4 g of crushed ice. The solid separated out was filtered, washed with ice-cold methanol and then with water. The crude product (**SCF1-8**) was recrystallized from rectified spirit.

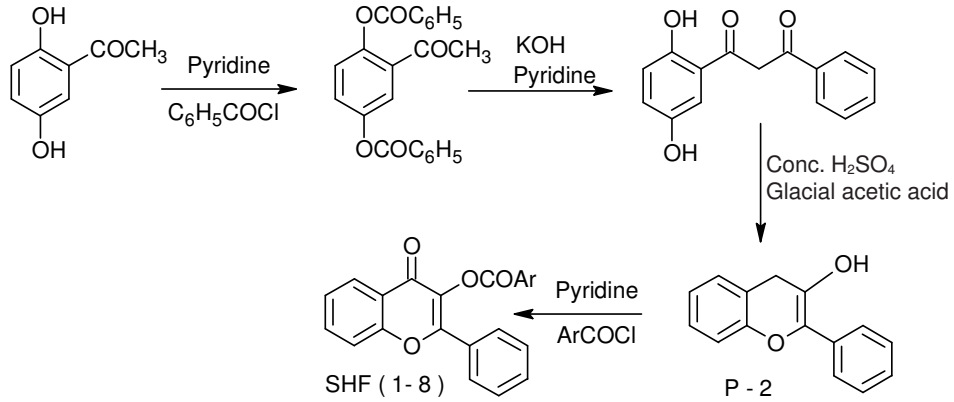


Scheme-I

Preparation of 2-phenyl esters of γ-benzopyrones (SHF series, Scheme-II): To (0.01mol) 2-phenyl-6-hydroxy-4-benzopyrone (**P-1**) and (0.014 mol) acyl acid chlorides, 2.5 mL of dry redistilled pyridine was added, shaken well and poured into 10 mL of 1 M HCl containing 4 g of crushed ice. The solid separated out was filtered, washed with ice-cold methanol and then with water. The crude product **(SHF1-8)** was recrystallized from rectified spirit.

Preparation of 2-phenyl esters of γ-benzopyrones (SDF series, Scheme-I): To (0.01 mol) 2-phenyl-3,6-dihydroxy-4-benzopyrone (**P-3**) and (0.014 mol) acyl acid chlorides, 2.5 mL of dry redistilled pyridine was added, shaken well and poured into 10 mL of 1 M HCl containing 4 g of crushed ice. The solid separated out was filtered, washed with ice-cold methanol and then with water. The crude product (**SDF1-8**) was recrystallized from rectified spirit.

Antioxidant activity: Antioxidant activity of the test compounds was determined by DPPH radical scavenging method as described earlier[6]. Different aliquots of test compound (10 to 1000 μg/mL) in methanol were mixed with 1 mM of methanolic DPPH˙ solution to a final volume of 2.0 mL, incubated for 20 min at room temperature and absorbance measured at 517 nm. Blank was carried-out in the same manner without the drug and ascorbic acid was taken as the standard.



Series			Ar
SHF	SCF	SDF	
SHF1	SCF1	SDF1	Phenyl
SHF2	SCF2	SDF2	*o*-Chlorophenyl
SHF3	SCF3	SDF3	*p*-Chlorophenyl
SHF4	SCF4	SDF4	*p*-Nitrophenyl
SHF5	SCF5	SDF5	*m*-Methylphenyl
SHF6	SCF6	SDF6	*p*-Methylphenyl
SHF7	SCF7	SDF7	*m*-Bromophenyl
SHF8	SCF 8	SDF8	Acetylphenyl

Scheme-II

Antimicrobial activity: Four strains of organism such as *Bacillus subtilis*, *Eshcerichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* were used for the determination of antimicrobial screening. The test compounds were initially screened for their qualitative antimicrobial activity by agar diffusion method[7,8]. The test compounds exhibiting promising activity were then evaluated for their MIC using 96 well plate method[9,10]. Gentamycin and amoxicillin were used as standard drugs.

Determination of minimum inhibitory concentration (MIC): The five isolates of microorganisms were exposed to graded concentrations (0.2-500 µg/50 µL) of the synthesized compounds and two standard antibiotics (gentamycin and amoxycillin) for 24 and 48 h. At the end of incubation the effect of the drugs on the growth of organisms were monitored by measuring the optical density at 540 nm using ELISA reader. The MIC was defined as the lowest concentration of the antibiotic or test sample allowing no visible growth. Determinations of MICs were performed in duplicate. The MIC for each of the compound was determined by plotting optical density (measure of bacterial growth) as a function of concentration of drugs.

RESULTS AND DISCUSSION

Synthesis and physico-chemical properties: The yield of the intermediate such as **P-1**, **P-2** and **P-3** were found to be 79.00, 76.00 and 67.00, respectively. The final compounds arrived upon treating the intermediates with different aryl acid chlorides in presence of pyridine gave compounds **SCF1-8**, **SDF1-8** and **SHF1-8** in the range of 59-79 %. The test compounds were purified by recrystallization using *n*-hexane and the purity was checked by melting point and TLC. The structure of the final compounds were established by IR, NMR and mass spectral studies (Tables 1-3).

TABLE-1
ACYL ESTERS OF γ-BENZOPYRONES (SCF SERIES)
PHYSICO-CHEMICAL PARAMETERS

Compd. code	m.f.	m.w.	Yield (%)	m.p. (ºC)	R_f value*	λ_{max} (nm)	log P	pKa
P1	$C_{15}H_9O_4Cl$	276.5	79	-	-	235	0.189	3.80
SCF1	$C_{22}H_{13}O_4$	376.5	72	109	0.36	226	0.239	5.17
SCF2	$C_{22}H_{12}O_4Cl_2$	411.0	75	122	0.40	241	0.317	4.18
SCF3	$C_{22}H_{12}O_4Cl_2$	411.0	70	143	0.44	241	0.299	-
SCF4	$C_{22}H_{12}NO_6Cl$	422.5	59	132	0.39	235	0.426	4.24
SCF5	$C_{23}H_{15}O_4Cl$	389.5	69	102	0.31	243	0.498	-
SCF6	$C_{23}H_{15}O_4Cl$	389.5	71	145	0.48	237	0.479	-
SCF7	$C_{22}H_{13}O_4BrCl$	454.5	70	178	0.50	237	0.319	5.23
SCF8	$C_{23}H_{15}O_4Cl$	389.5	66	141	0.60	285	0.219	7.29

*Benzene : chloroform (1:5).

TABLE-2
ACYL ESTERS OF γ-BENZOPYRONES (SHF SERIES)
PHYSICO-CHEMICAL PARAMETERS

Compd. code	m.f.	m.w.	Yield (%)	m.p. (ºC)	R_f value*	λ_{max} (nm)	log P	pKa
P2	$C_{15}H_{12}O_2$	224.2	76	-	-	227	0.178	7.90
SHF1	$C_{22}H_{14}O_4$	342.0	61	110	0.53	256	0.228	8.30
SHF2	$C_{22}H_{13}O_4Cl_1$	376.5	75	104	0.54	262	0.289	-
SHF3	$C_{22}H_{12}O_4Cl_2$	376.5	70	156	0.34	237	0.302	11.78
SHF4	$C_{22}H_{12}NO_6Cl$	388.0	66	129	0.59	281	0.308	11.03
SHF5	$C_{23}H_{15}O_4Cl$	356.0	74	135	0.41	248	0.528	11.36
SHF6	$C_{23}H_{15}O_4Cl$	356.0	64	114	0.38	279	0.492	-
SHF7	$C_{22}H_{13}O_4BrCl$	421.0	67	198	0.65	282	0.312	-
SHF8	$C_{23}H_{15}O_4Cl$	356.0	70	140	0.36	281	0.190	11.81

*Benzene : chloroform (4:2).

log P values: Partition coefficients of the synthesized compounds were determined by the classical shake flask method using *n*-octanol and phosphate buffer of pH 7.4. The Tables 1-3 indicate that the log P values for the compounds synthesized (**SCF1-8**, **SHF1-8** and **SDF1-8**). Presence of the electron-withdrawing group on

TABLE-3
ACYL ESTERS OF γ-BENZOPYRONES (SDF SERIES)
PHYSICO-CHEMICAL PARAMETERS

Compd. code	m.f.	m.w.	Yield (%)	m.p. (ºC)	R_f value*	λ_{max} (nm)	log P	pKa
P3	$C_{15}H_{10}O_4$	254	67	-	-	227	0.138	6.90
SDF1	$C_{29}H_{18}O_6$	462	65	115	0.45	254	0.278	8.30
SDF2	$C_{29}H_{16}O_6Cl_2$	532	68	136	0.58	243	0.307	8.17
SDF3	$C_{29}H_{16}OCl_2$	532	69	165	0.64	307	0.310	-
SDF4	$C_{29}H_{16}N_2O_{10}$	553	68	180	0.53	270	0.422	8.33
SDF5	$C_{31}H_{20}O_6$	490	60	105	0.62	241	0.419	-
SDF6	$C_{31}H_{20}O_6$	490	71	122	0.49	273	0.404	-
SDF7	$C_{29}H_{16}O_6Br_2$	620	66	118	0.74	-	-	-
SDF8	$C_{31}H_{20}O_6$	490	65	169	0.39	-	-	-

*Benzene : chloroform (1:1).

6th position of the γ-benzopyrone ring significantly influenced the partition coefficient of the parent compound **P-1**. Parent compound **P-1** with chloro substitution on 3rd position and hydroxyl group on 6th position of the ring showed greater log P values at 0.189 than that of the parent compounds **P-2** at 0.178 and **P-3** at 0.138 with no substitution on 6th position of the γ-benzopyrone ring. The order of decrease in polarity among the three parent compounds is 3-hydroxy-6-chloro flavones < 6-hydroxy flavone < 3,6-dihydroxy flavones. Out of 24 test compounds synthesized, compounds with chloro substitution on γ-benzopyrone ring had greater log P values (**SCF1-8**) when compared to that of test compounds without chloro substitution on the ring (**SHF1-8** and **SDF1-8**) as shown in the Tables 1-3. These observations clearly indicated that, the polarity of the compounds decreases with chloro substitution on the ring. However, the decrease in polarity among the three series of compounds synthesized were in the order of **SCF1-8 < SHF-1-8 < SDF-1-8**.

Further, the effects of electron withdrawing and donating groups were attempted on the aromatic ring derived from the corresponding acid chlorides. There was a significance drop in the log P values irrespective of the nature the substituent group on the ring. The positioning of the substituent groups was also studied. Nitro group at *p*-position on the aromatic ring was showing higher log P value compared with other positions. The presence of electron donating group such as methyl group on the aromatic ring showed increased log P value as shown in the Tables 1-3. However, log P values for **SDF7-9** could not be determined due to solubility problems encountered in *n*-octanol. Thus, decreasing order of polarity for the substituents on the aromatic nucleus can be given as, *p*-tolyl < *m*-tolyl < *p*-nitro < *m*-bromo < *p*-chloro < *o*-chloro < phenyl < benzyl substitution.

pKa Values: pH of the parent compounds **P1-3** in their saturated solutions were found to be at 2.2, 3.4 and 2.3, respectively with their pKa values **P1** (3.8), **P2** (7.9) and **P3** (6.9). From the experimental data, it was known that all the parent compounds were acidic in nature. Out of 24 compounds synthesized, few compounds

encountered solubility problems in ethanol and water. Thus, test compounds that were soluble in ethanol were taken for their pKa studies. All the test compounds showed greater pKa values than their corresponding parent compounds. The acidic nature of the test compounds may be owing to the presence of phenolic hydroxy group. It was noted that the test compounds from **SCF** series exhibited pKa in the range of 3 to 5.5. Whereas, test compounds from **SHF** and **SDF** series showed pKa in the range from 8 to 11.8 and 8 to 8.3, respectively. The pKa values of the parent compounds and the test compounds are shown in Tables 1-3.

Spectral data

6-Chloro-3-hydroxy-2-phenyl-4-benzopyrone (P1): IR (KBr, ν_{max}, cm^{-1}): 3145 (b, O–H *str.*), 3058 (s, Ar C–H *str.*), 2362 (w, C–H *str.*), 1697 (s, C=O *str.*), 1575 and 1498 (s, Ar C–C *str.*), 1396 (s, C–O *str.* and O–H def). ^{1}H NMR: 8.11 (s, 1H, O–H) 7.46-7.50 (m, 5H, Ar–H) 7.60-7.64 (m, 3H, Ar–H).

6-Hydroxy-2-phenyl-4-benzopyrone (P2): IR(KBr, ν_{max}, cm^{-1}): 3249 (b, O–H *str.*), 3058 (s, Ar C–H *str.*), 2364 (w, C-H *str.*), 1678 (s, C=O *str.*), 1575 and 1591 (s, Ar C–C *str.*), 1402 (s, C–O *str.* and O-H def). ^{1}H NMR: 8.70 (s, 1H, Ar-H), 7.4-7.7 (m, 3H, Ar–H), 7.3-7.96 (m, 5H, Ar–H).

6′-Chloro-2′-phenyl-4′-benzopyrone-3′-yl-4-chlorobenzoate (SCF_3): IR (KBr, ν_{max}, cm^{-1}): 3079 (s, Ar C–H *str.*), 2841 (w, C–H *str.*), 1787.9 (s, C=O *str.* of ester), 1687 (s, C=O *str.* of lactone), 1550 and 1490 (s, Ar C–C *str.*), 1396 (s, C–O *str.*) 709 (s, C–Cl *str.*) Mass: Base peak at m/e = 119 Other peaks were observed at m/e = 269, 254, 226, 120, 105, 91, 77, 65, 50, 44. ^{1}H NMR: 7.44-8.08 (m, 5H, Ar–H), 7.44-7.46 (m, 2H, Ar-H), 8.02-8.04 (m, 2H, Ar–H).

2′-Phenyl-4′-benzopyrone-6′-yl-4-methylbenzoate (SHF6): IR (KBr, ν_{max}, cm^{-1}): 3076 (s, Ar C–H *str.*), 2362 (w, C–H *str.*), 1735 (s, C=O *str.* of ester), 1689 (s, C=O *str.* of lactone), 1593 and 1498 (s, Ar C–C *str.*), 1396 (s, C–O *str.*), Mass: Base peak at m/e = 139 (M^+), 141 (M^{+2}) Other peaks were observed at m/e = 160, 156 (M^+), 158 (M^{+2}), 111(M^+), 113 (M^{+2}), 83, 77, 65, 50, 44. ^{1}H NMR: 2.42-2.45 (s, 3H, C–H) 7.30-7.32 (m, 2H, Ar–H) 8.02-8.04 (m, 2H, Ar–H) 7.50-8.16 (m, 5H, Ar–H) 7.51 (s, 1H, Ar–H).

Antioxidant activity: The antioxidant activity of the compounds was done by DPPH$^{\wedge}$ method. EC_{50} of all the selected test compounds and ascorbic acid as reference standard are reported in the Tables 4-6. Out of the 24 test compounds from three different parents and the parent compounds tested for their antioxidant property, compounds such as **SCF2**, **SDF5**, **SDF6**, **SDF7** and all the three parent compounds showed significant free radical scavenging activity with respect to DPPH$^{\wedge}$. However, compound **P-2** showed the highest antiradical property *in vitro*. Thus, from the study, it was found that **P-2** was the only potential candidate for scavenging radical oxygen as its EC_{50} was found to be 40 μg/mL calculated using Microsoft excel.

Antimicrobial activity

Qualitative antimicrobial activity studies (using agar diffusion method): All the 24 test compounds synthesized, purified and characterized were screened

TABLE-4
ANTIOXIDANT ACTIVITY STUDIES (**SCF SERIES**)

µg/mL*	**P1**	**SCF1**	**SCF2**	**SCF3**	**SCF4**	**SCF5**	**SCF6**	**SCF7**	**SCF8**
0	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900
20	0.819	1.061	0.986	0.98	0.981	1.140	1.145	0.974	0.901
40	0.812	1.281	0.901	0.92	0.965	0.854	1.256	0.865	0.908
60	0.807	1.327	0.877	0.987	1.12	1.085	0.968	0.976	0.869
80	0.625	1.182	0.826	1.221	0.958	1.055	0.942	0.932	0.919
100	0.612	1.136	0.806	0.965	0.997	0.993	0.922	1.08	0.886

*Concentration of the test compounds.

TABLE-5
ANTIOXIDANT ACTIVITY STUDIES (**SHF SERIES**)

µg/mL*	**P2**	**SHF1**	**SHF2**	**SHF3**	**SHF4**	**SHF5**	**SHF6**	**SHF7**	**SHF8**
0	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900
20	0.782	1.130	1.261	0.905	0.981	0.898	0.980	1.042	0.800
40	0.526	1.055	1.321	0.892	1.280	0.968	0.987	0.983	0.980
60	0.499	1.252	1.114	0.725	0.906	0.956	0.915	0.856	0.954
80	0.467	1.315	1.121	0.711	0.869	0.768	0.948	0.895	0.901
100	0.321	0.967	1.630	0.692	0.789	1.120	0.876	0.982	0.768

*Concentration of the test compounds.

TABLE-6
ANTIOXIDANT ACTIVITY STUDIES (**SDF SERIES**)

µg/mL*	**P3**	**SDF1**	**SDF2**	**SDF3**	**SDF4**	**SDF5**	**SDF6**	**SDF7**	**SDF8**
0	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900	0.900
20	0.842	1.227	1.212	0.908	1.150	0.922	0.875	1.130	0.983
40	0.764	1.108	0.982	0.856	1.125	0.915	0.981	1.254	0.852
60	0.616	1.288	1.413	0.812	1.061	0.897	0.928	1.020	0.951
80	0.532	1.330	1.312	0.801	0.985	0.887	0.973	0.865	0.975
100	0.414	1.117	1.126	0.762	0.867	0.898	0.987	0.966	1.570

*Concentration of the test compounds.

for their qualitative antimicrobial activity by agar diffusion method. They were tested against four species of bacteria namely, *Bacillus subtilis* (Gram-positive), *Escherichia coli* (Gram-negative), *Klebsiella pneumonia* (Gram-negative), *Staphylococcus aureus* (Gram-positive).

Compounds such as **SCF3**, **SCF7**, **SCF8**, **SHF4**, **SDF1**, **SDF2** and **SDF4** showed activity against both Gram-positive bacterias and were found to have zones of inhibition values at 16, 17, 18, 15, 15, 17 and 16 mm, respectively against *Bacillus subtilis* and 18, 19, 17, 17, 14, 17 and 18 mm, respectively against *Staphylococcus aureus*. However, none of the compounds showed activity against Gram-negative bacteria, presumably due to their polar nature. The compounds tested for their antimicrobial activity with their zones of inhibition and log P values are shown in the Tables 7-9.

TABLE-7
ANTIMICROBIAL ACTIVITY CHART OF **SCF**, **SHF** AND **SDF** SERIES AGAINST GRAM-POSITIVE ORGANISMS *Bacillus subtilis* AND *Staphylococcus aureus*

Compd.	Zone of inhibition (mm)		Compd.	Zone of inhibition (mm)		Compd.	Zone of inhibition (mm)	
	B. subtilis	*S. aureus*		*B. subtilis*	*S. aureus*		*B. subtilis*	*S. aureus*
SCF1	11	-	**SHF1**	-	08	**SDF1**	15	14
SCF2	14	-	**SHF2**	8	11	**SDF2**	17	17
SCF3	16	18	**SHF3**	-	12	**SDF3**	12	13
SCF4	-	11	**SHF4**	15	17	**SDF4**	16	18
SCF5	10	13	**SHF5**	10	12	**SDF5**	10	12
SCF6	-	11	**SHF6**	-	11	**SDF6**	11	9
SCF7	17	19	**SHF7**	-	9	**SDF7**	9	-
SCF8	18	17	**SHF8**	11	-	**SDF8**	-	-
Std.1	31	27	Std 1	31	26	Std 1	30	28
Std.2	35	32	Std 2	34	30	Std 2	35	32

Std.1: Amoxycillin; Std.2: Gentamycin.

TABLE-8
ANTIMICROBIAL ACTIVITY DATA FROM ELISA FOR *Staphylococcus aureus*

Compound	MIC (µg/µL)	% Inhibition as compared to gentamycin	% Inhibition as compared to amoxycillin
SCF3	102	32	30
SCF7	51	43	54
SCF8	-	-	-
SHF4	-	-	-
SDF1	102	35	43
SDF2	102	41	38
SDF4	-	-	-
Amoxycillin	102		
Gentamycin	51		

TABLE-9
ANTIMICROBIAL ACTIVITY DATA FROM ELISA FOR *Bacillus subtilis*

Compound	MIC (µg/µL)	% Inhibition as compared to gentamycin	% Inhibition as compared to amoxycillin
SCF3	51	60	65
SCF7	25	64	69
SCF8	-	-	-
SHF4	102	57	61
SDF1	25	64	68
SDF2	102	56	61
SDF4	-	-	-
Amoxycillin	102		
Gentamycin	51		

Quantitative antimicrobial activity studies (Using two fold dilution technique): Out of the 24 test compounds screened for qualitative antibacterial activity by Agar diffusion method, 7 compounds such as **SCF3**, **SCF7**, **SCF8**, **SHF4**, **SDF1**, **SDF2** and **SDF4** showed activity against Gram-positive bacteria. It was found worthwhile to evaluate their antibacterial activity quantitatively. Thus, two fold dilution technique using ELISA reader was used to measure their minimum inhibitory concentration (MIC). The organisms selected were *Staphylococcus aureus* and *Bacillus subtilis*. Amoxycillin and gentamycin were selected as standards in the concentration range 0.2-500 µ/mL. The test compounds and standards were prepared in DMSO and the solutions were further diluted with DMSO. The concentration of DMSO used for the purpose of dilution however, did not show any antibacterial activity.

The test compounds from **SCF** series such as **SCF3** and **SCF7** showed MIC at 102 and 51 µg/µL, respectively for *Staphylococcus aureus* and 51 and 25 µg/µL, respectively for *Bacillus subtilis*. The percentage inhibition of **SCF-3** and **SCF-7** were found at 32:30 % and 43:54 % against *Staphylococcus aureus* using amoxycillin and gentamycin as standards, respectively. However, the % inhibition of **SCF3** and **SCF7** were found at 60:64 % and 65:69 % against *Bacillus subtilis* using the same standard antibiotics. The results are shown in Tables 8 and 9. The test compound **SHF4** showed MIC at 102 µg/µL for *Bacillus subtilis* with % inhibition at 57 and 61 %. Both the test compounds **SDF1** and **SDF2** showed MIC at 102 µg/µL against *Staphylococcus aureus* and 25 and 102 µg/µL, respectively against *Bacillus subtilis*. The percentage inhibition of **SDF1** and **SDF2** were found at 35:31 % and 41:38 % against *Staphylococcus aureus* using amoxycillin and gentamycin as standards, respectively. However, the percentage inhibition of **SDF3** and **SDF7** were found at 64:68 % and 56:61 % against *Bacillus subtilis* using the same standard antibiotics. The results are shown in Tables 8 and 9.

The test compounds possessing halogen substitution such as chloro and bromo substituents on the aromatic ring showed higher activity than the groups such as hydrogen, methyl and nitro. Eventhough, the log P value for the nitro-substituted compound was greater than other counterparts, the order of antibacterial activity was expressed as bromo substituted > chloro substituted > nitro substituted > unsubstituted compounds.

Conclusions

log P values obtained for the test compounds were in the range of 0.190 to 0.528 and the pKa values ranges from 4.18 to 11.81. 15 compounds showed appreciable DPPH radical scavenging activity. Seven compounds showed activity against Gram-positive bacteria. Their activity was quantitatively measured by two-fold dilution technique using ELISA reader. All the compounds showing antioxidant activity also showed antibacterial activity and thus the relationship between log P, antioxidant and antibacterial activity were established in present study.

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