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Synthesis and anti-cancer, anti-metastatic evaluation of some new fluorinated isocoumarins and 3,4-dihydroisocoumarins

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ABSTRACT

Synthesis of some fluorinated isocoumarins and 3,4-dihydroisocoumarins is reported. Structures of the synthesized compounds were confirmed by spectral and elemental analysis. All the synthesized compounds were evaluated for their antimetastatic activity and anti-cancer activity against breast cell line (MCF-7). Among all the tested compounds **3h** [3-(3′,4′-difluorophenyl)isocoumarin] has shown excellent antimetastatic activity, however the growth inhibitory response of the tested compounds [**3a–h**, **4a–h**, **5e–h**, **6a–h** and **7a**, **c**, **d**] for MCF-7 human breast carcinoma cell line was moderate at higher concentrations (100–400 μM) and negligible at lower concentrations.

1. Introduction

Metastasis is the most deadly aspect of cancer in which it spreads from its primary site to other places of the body. This process involves a series of tightly coupled events. These include the detachment of tumor cells from the primary site, migration of the cells accompanied by regulated proteolysis of the extracellular matrix/basement membrane, dissemination of the tumor cells through the vasculature and finally adhesion and proliferation of the cells at a secondary site.

Isocoumarins and 3,4-dihydroisocoumarins are a group of biologically active compounds that have been shown to influence not only hormone metabolism but also intracellular enzymes [1], protein synthesis, growth factors, malignant cell proliferation and angiogenesis [2,3]. These compounds have been successfully evaluated for antifungal, antibacterial, anti-tumor or cytotoxic activities [4–6]. Rossi et al. [7] prepared different 3-aryliso-couamrins which showed cytotoxic activity against human cancer cell lines *in vitro*. Queiroz et al. [8] prepared different tricyclic lactones which showed a high growth inhibitory effect (*in vitro*) on the growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer) and SF-268 (CNS cancer). In a study [9], it was found that bireticulol has cytotoxic effects against KB (human epidermoid carcinoma, ATCC CCL-17) and NCI-H187 (human small cell lung cancer, ATCC CRL-5804) cell lines with IC_{50} values of 24.4 and 8.31 μg mL^{-1} respectively. In another study [10], an antiangiogenic isocoumarin, NM-3, has been found to increase the antitumor effects of radiotherapy without toxicity. NM-3 alters several stages of the angiogenic process including endothelial cell survival, migration, and tube formation.

Organic compounds with fluorine moiety are medicinally very important [11]. Drug-receptor interactions are improved in the presence of small sized highly electronegative fluorine atom. The transport of drug is facilitated by the high lipophilicity of organofluorine compounds [12]. The literature survey revealed that among the halogenated isocoumarins, tested for anticancer activities, fluorinated isocoumarins have been rarely screened. So in continuation of our previous studies [13–21], here we report the synthesis of some new fluorine containing isocoumarins and their conversion to the corresponding dihydroisocoumarins in order to check their antimetastatic activity and anti-cancer activity against breast cell line (MCF-7).

General synthetic scheme is shown as follows (Scheme 1).

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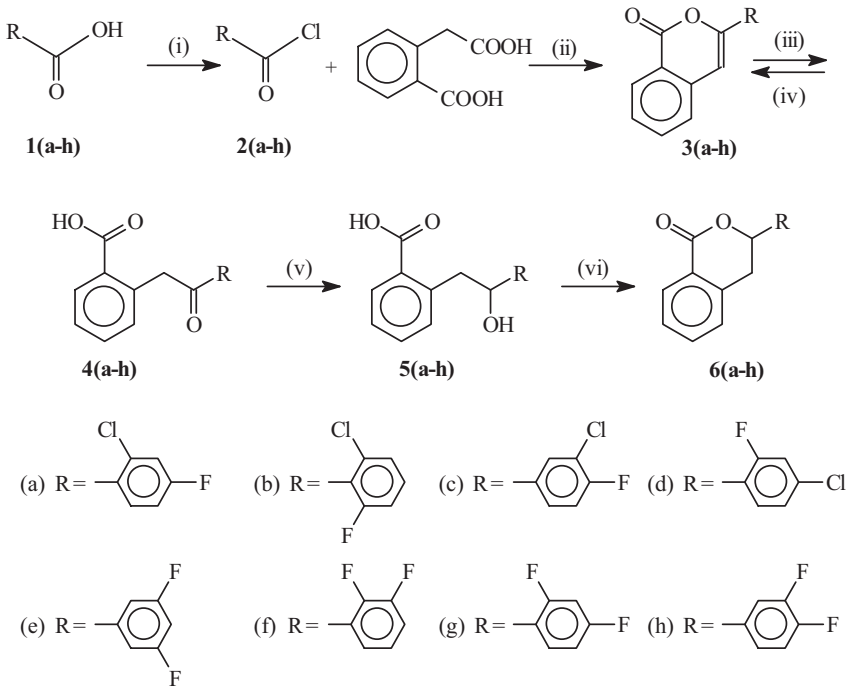
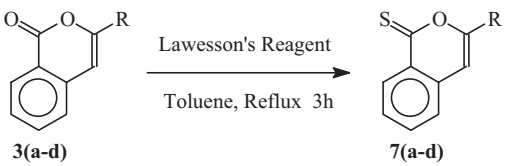


Scheme 1. Synthesis of dihalophenylisocoumarins: (i) $SOCl_2$, 60 °C for 30 min; (ii) reflux 4 h; (iii) 5% KOH/ethanol, reflux 5 h; (iv) Ac_2O, reflux 1 h; (v) 1% NaOH/$NaBH_4$, overnight stirring (vi) Ac_2O, reflux 1 h.

2. Chemistry

Synthesis of the target compounds is shown in the general synthetic Schemes 1 and 2. Condensation of the acid chloride with homophthalic acid is a useful method for the preparation of 3-substituated isocoumarins skeleton [22]. A short and efficient synthesis of 3-(dihalophenyl)isocoumarins **3(a–d)** using this method and conversion of **3(a–d)** into corresponding racemic 3-(dihalophenyl)-3,4-dihydroisocoumarins **6(a–d)** were achieved. Dihalobenzoic acids **1(a–d)** were converted into their respective acid chlorides **2(a–d)** by reaction with thionyl chloride. Direct condensation of acid chlorides **2(a–d)** with homophthalic acid at 200 °C afforded 3-dihalophenylisocoumarins **3(a–d)** in 63–73% yield, which were purified by column chromatography. These isocoumarins **3(a–d)** exhibited characteristic 1H-singlet at δ 6.70–7.20 ppm for C_4–H in ^{1}H NMR, while in IR spectra lactonic carbonyl absorptions were observed at 1716–1728 cm^{-1}. Alkaline hydrolysis of isocoumarins **3(a–d)** afforded 2-(dihalobenzoylmethyl)benzoic acids **4(a–d)**. Isocoumarins **3(a–d)** were obtained back on refluxing keto-acids **4(a–d)** with acetic anhydride. Sodium borohydride reduction of keto-acids **4(a–d)** afforded the corresponding racemic hydroxy acids **5(a–d)**, which were cyclodehydrated with acetic anhydride to produce 3-dihalophenyl-3,4-dihydroisocoumarins **6(a–d)** which exhibited the carbonyl absorptions at 1712–1731 cm^{-1} in IR spectra. The typical AB pattern for C_3–H proton and ABX pattern for C_4–H protons were observed in ^{1}H NMR spectrum of the compound **6(a-d)**. Thus, each of the C_4–H showed a doublet of doublet at δ 3.01–3.18 ppm and δ 3.27–3.48 ppm and another doublet of doublet was observed at δ 5.48–6.15 ppm for C_3-H proton. Thioisocoumarins **7(a–d)** were synthesized by thionation of isocoumarins **3(a–d)** by refluxing with Lawesson's reagent [23] as shown in Scheme 2 and the structures were confirmed by the appearance of new peaks at 1061–1109 cm^{-1} for C=S in IR. The structure elucidations of newly synthesized compounds were determined by modern spectroscopic techniques like IR, ^{1}H NMR and ^{13}C NMR. Further confirmations of the compounds were carried out by mass spectrometry and microanalysis. We have already reported synthesis, anti-oxidant, and anti-analgesic activities of compounds (**3e–h**, **4e–h**, **5e–h** and **6e-h**) [20]; however in this paper we want to report their anti-cancer and anti-metastatic activities.



Scheme 2. Conversion of isocoumarins to thioisocoumarins.

3. Pharmacology

3.1. Antimetastatic activity and anticancer activity against breast cell line MCF-7

3.1.1. Cell culture

MCF-7/AZ is a variant of the human mammary carcinoma cell family MCF-7 [24]. The cells are maintained on a tissue culture plastic substrate (Nunc) in a mixture of Dulbecco's modified Eagle's medium (DMEM) and HAMF12 (50/50) (Invitrogen, Carlsbad, CA, USA) supplemented with 250 IU/mL penicillin, 100 μg mL^{-1} streptomycin (Invitrogen) and 10% fetal bovine serum (FBS) (Invitrogen), at 37 °C in a humidified atmosphere containing 10% CO_2.

3.1.2. Assay for cell viability

Thirty-one compounds (**3a–h**, **4a–h**, **5e–h**, **6a–h** and **7a**, **c**, **d**) were evaluated for their anticancer activities against MCF-7 breast cell line. Cell viability was tested in accordance with Romijn et al. [25]. Briefly mitochondrial dehydrogenase activities were measured by an MTT-reagent (Sigma, St. Louis, MO, USA). Cells were seeded in microtiter plates at an initial density of 1.5×10^4 cells in 200 μL culture medium and treated with increasing concentrations of each

compound. In each experiment, eight wells were used to determine the mean optical density (OD) referring to cell viability.

3.1.3. Collagen type I invasion assay

This was performed as described [26]. Briefly, six-well plates were filled with 1.25 mL neutralized type I collagen (0.09%) (Upstate Biotechnology, Lake Placid, NY) and incubated for 1 h at 37 °C to allow gelification. Non-invasive MCF-7/Az cells were pretreated with ET-18-OMe for 24 h in order to become invasive into collagen type I [27] and served as control for invasiveness as compared to untreated MCF-7/Az cells. Single cell suspensions were prepared with trypsin/EDTA, mixed with different compound solutions, seed on top of collagen type I gel and cultured at 37 °C for 24 h. The number of cells penetrating into the gel or remaining at the surface were counted in 12 field of 0.157 mm^2 [27], using an inverted microscope controlled by a computer programme. This invasion index expresses the percentage of invading cells over the total number of cells.

4. Results

4.1. Anti-metastatic activity

Thirty one compounds (**3a–h**, **4a–h**, **5e–h**, **6a–h** and **7a**, **c**, **d**) were evaluated for their antimetastatic activities. The results for inhibition of invasion of these cells into collagen, which is an indication of antimetastatic activity are given in Table 1. All the tested compounds showed very small antimetastatic activity except the compound **3h** [3-(3′,4′-difluorophenyl)isocoumarin] which has shown excellent activity. This activity may be attributed to the presence of two fluoro groups at meta and para positions of the phenyl ring attached at C-3 in conjugation with the double bond of isocoumarin nucleus.

Table 1
Anti-metastatic activity of the synthesized compounds (**3a–h**), (**4a–h**), (**5e–h**), (**6a–h**) and (**7a, c–d**).

Compd.	Collagen type I invasion	Treatment corresponding OD80 cells MCF-7/AZ
3a	16	2
3b	15	2
3c	16	1
3d	13	3
3e[a]	17	3
3f[a]	16	2
3g[a]	16	2
3h[a]	2	1
4a	15	2
4b	19	2
4c	18	2
4d	16	2
4e[a]	17	2
4f[a]	16	2
4g[a]	16	1
4h[a]	18	2
5e[a]	18	3
5f[a]	17	2
5g[a]	18	2
5h[a]	18	3
6a	18	1
6b	17	2
6c	17	2
6d	18	1
6e[a]	18	1
6f[a]	16	2
6g[a]	16	2
6h[a]	16	1
7a	15	2
7c	14	1
7d	10	1
+control	19	1

[a] Synthesis of these compounds is reported already [22].

4.2. Anti-cancer activity against breast cell line (MCF-7)

Growth inhibitory effect of the tested compounds against human breast carcinoma cell line MCF-7 is shown in Table 2. It is clear from the table that the inhibition is moderate at higher concentrations (100–400 μM) however it is negligible at lower concentrations (<100 μM).

Table 2
Anticancer activity against breast cell line (MCF-7) of synthesized compounds (**3a–h**), (**4a–h**), (**5e–h**), (**6a–h**) and (**7a**, **c–d**).

Compd.		Concentration (μM)					
		0	2.5	25	50	100	400
3a	% viability	100	98	91	89	83	79
	% std		5	1	6	4	5
3b	% viability	100	112	110	104	95	84
	% std		3	5	6	8	6
3c	% viability	100	97	96	95	94	89
	% std		7	6	5	6	4
3d	% viability	100	91	80	82	80	75
	% std		9	6	11	7	10
3e[a]	% viability	100	108	118	112	60	53
	% std		6	2	7	6	10
3f[a]	% viability	100	104	90	87	77	48
	% std		6	2	8	4	8
3g[a]	% viability	100	92	82	80	80	68
	% std		5	8	8	4	4
3h[a]	% viability	100	75	80	82	70	62
	% std		7	6	11	7	9
4a	% viability	100	104	100	97	77	73
	% std		6	3	8	4	6
4b	% viability	100	92	82	80	80	78
	% std		5	8	8	4	2
4c	% viability	100	98	93	90	90	88
	% std		6	5	5	6	7
4d	% viability	100	113	111	99	91	89
	% std		6	8	5	4	8
4e[a]	% viability	100	104	90	87	77	70
	% std		6	2	8	4	8
4f[a]	% viability	100	92	82	80	80	75
	% std		5	8	8	4	4
4g[a]	% viability	100	98	95	91	90	82
	% std		7	5	7	6	8
4h[a]	% viability	100	103	98	92	91	89
	% std		4	8	9	4	8
5e[a]	% viability	100	93	93	88	80	79
	% std		7	8	9	6	2
5f[a]	% viability	100	91	90	89	86	48
	% std		4	8	4	3	5
5g[a]	% viability	100	89	86	86	75	71
	% std		4	9	7	9	8
5h[a]	% viability	100	100	91	90	85	83
	% std		8	3	5	7	8
6a	% viability	100	105	104	92	80	80
	% std		7	5	7	6	4
6b	% viability	100	98	92	89	86	76
	% std		5	8	6	3	8
6c	% viability	100	99	98	86	81	79
	% std		4	5	7	8	4
6d	% viability	100	100	97	90	89	83
	% std		8	4	5	6	8
6e[a]	% viability	100	96	93	82	83	77
	% std		5	6	7	5	9
6f[a]	% viability	100	93	93	90	86	85
	% std		8	4	7	7	7
6g[a]	% viability	100	75	80	82	83	80
	% std		7	6	8	5	9
6h[a]	% viability	100	92	85	80	79	71
	% std		5	8	8	4	4
7a	% viability	100	96	93	82	83	77
	% std		5	6	7	5	9
7c	% viability	100	97	95	90	78	77
	% std		6	4	7	8	7
7d	% viability	100	95	80	82	80	77
	% std		6	6	8	5	6

[a] Synthesis of these compounds is reported already [22].

5. Conclusion

A total of thirty one compounds {fifteen new (**3a–d**, **4a–d**, **6a–d**, **7a**, **7c** and **7d**) and sixteen already reported (**3e–h**, **4e–h**, **5e–h**, **6e–h**) by us [22]} were screened for anti-cancer and anti-metastatic activities. These compounds have shown MCF-7 human breast cancer cell inhibition activity at higher concentrations. Antimetastatic activity of the tested compounds was small except the compound **3h** which has surprisingly shown excellent activity and it is far better than the control. The compound **3h** may be a potential lead candidate with respect to antimetastatic activity and for further biological exploration.

6. Experimental

All the common chemicals and solvents were of analytical grade or dry distilled. All the processes involving air or moisture sensitivity were conducted under the inert atmosphere of dry nitrogen using oven dried glassware. The solvent evaporation was performed under reduced pressure using a Büchi rotary evaporator. Melting points were determined on Stuart melting point apparatus (SMP3) and are uncorrected. The IR spectra were recorded on Bio-Rad Merlin FTS 3000 MX spectrophotometer. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded on a Bruker AM-300 as DMSO and $CDCl_3$ solutions using TMS as internal standard. Chemical shifts (δ) are expressed in units of parts per million relative to TMS. Electron impact mass spectra (EIMS) were recorded on a Finnigan MAT-311A Germany. CHN analyses were performed on a Carlo Erba Strumentazion-Mod-1106 Italy. Thin layer chromatography was performed on pre-coated silica gel plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by, UV at 254 and 365 nm. The synthetic pathway for the compounds is shown in Schemes 1 and 2.

6.1. General synthetic procedure for the isocoumarin derivatives 3(a–d)

2-Chloro-4-fluorobenzoic acid, 2-chloro-6-fluorobenzoic acid, 3-chloro-4-fluorobenzoic acid and 4-chloro-2-fluorobenzoic acid **1(a–d)** (5 g, 28.7 mmol) were converted into their respective acid chlorides **2(a–d)** by reaction with thionyl chloride (4.09 g, 2.49 mL and 34 mmol) in the presence of a drop of DMF under reflux for 30 min. Completion of the reaction was indicated by the stoppage of gas evolution. Removal of excess of thionyl chloride was carried out under reduced pressure to afford dihalobenzoyl chlorides **2(a–d)**. Mixtures of homophthalic acid (1.3 g, 7.2 mmol) and dihalobenzoyl chlorides **2(a–d)** (5.516 g, 28.7 mmol) were heated at 200 °C under reflux for 4 h. The mixtures were dissolved in ethyl acetate and aqueous solution of sodium carbonate was added in order to remove the unreacted homophthalic acid. Organic layer was separated and the aqueous layer was extracted with 2× 25 mL ethyl acetate. The combined organic layers were concentrated and chromatographed on silica gel using pet ether (40–80 °C fraction) as eluent which gave 3-(dihalophenyl)isocoumarins **3(a–d)** as solids, which were further purified by recrystallization from methanol.

6.1.1. 3-(2′-Chloro-4′-fluorophenyl)isocoumarin (3a)

Yield 68%; m.p. 163–164 °C; IR (KBr) ν_{max} in cm^{-1}: 1728 (C=O), 1117 (C–F), 1069 (C–Cl); ^{1}H NMR ($CDCl_3$, δ-values): 7.20 (1H, s, H-4), 7.21–7.30 (2H, m, H-3′,5′), 7.52–7.58 (2H, m, H-5,6′), 7.76 (1H, dt, *J* = 7.5, 1.2 Hz, H-7), 7.98 (1H, dt, *J* = 7.5, 1.2 Hz, H-6), 8.33 (1H, dd, *J* = 8.1, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 103.9 (C-4), 114.4 (d, *J* = 22.5 Hz, C-5′), 118.0 (d, *J* = 22.5 Hz, C-3′), 120.6 (C-5), 128.4 (d, *J* = 3.9 Hz, C-1′), 129.5 (C-7), 129.8 (C-8a), 131.2 (d, *J* = 9.0 Hz C-6′), 133.4 (C-8), 134.6 (d, *J* = 9.0 Hz, C-2′), 136.8 (C-6, 4a), 148.2 (C-3), 156.5 (C-1), 162.2 (d, *J* = 252 Hz, C-4′) ppm; MS (EI, 70 eV): *m*/*z* (%); =274 ($M^{+\bullet}$, 85), 246 (93.5), 227 (2.6), 211 (100), 157 (24.3), 145 (1.4), 117 (4.2), 89 (37.4); Elemental Analysis: Found (Calcd.)%; C: 65.60 (65.01), H: 2.91 (2.85).

6.1.2. 3-(2′-Chloro-6′-fluorophenyl)isocoumarin (3b)

Yield 69%; m.p. 240–241 °C; IR (KBr) ν_{max} in cm^{-1}: 1720 (C=O), 1182 (C–F), 1047 (C–Cl); ^{1}H NMR ($CDCl_3$, δ-values): 6.70 (1H, s, H-4), 7.12 (1H, dt, *J* = 8.1, 3.0 Hz, H-4′), 7.28–7.43 (2H, m, H-3′,5′), 7.52 (1H, d, *J* = 7.8 Hz, H-5), 7.59 (1H, dt, *J* = 7.8, 1.2 Hz, H-7), 7.78 (1H, dt, *J* = 7.5, 1.2 Hz, H-6), 8.37 (1H, dd, *J* = 7.8, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 102.0 (d, *J* = 2.4 Hz, C-4), 111.7 (d, *J* = 21.9 Hz, C-5′), 120.3 (d, *J* = 21.9 Hz, C-1′), 124.2 (C-5), 126.2(d, *J* = 3.0 Hz, C-3′), 128.9 (C-7), 129.8 (C-8, 8a), 130.3 (d, *J* = 8.4 Hz, C-4′), 133.1 (d, *J* = 7.4 Hz, C-3), 133.9 (C-6, 4a), 136.5 (d, *J* = 8.4 Hz, C-2′), 157.2 (C-1), 161.4 (d, *J* = 249 Hz, C-6′) ppm; MS (EI, 70 eV): *m*/*z* (%); =274 ($M^{+\bullet}$, 85), 246 (90.7), 227 (3.5), 211 (100), 157 (22.4), 145 (2.5), 117 (3.5), 89 (35.9); Elemental Analysis: Found (Calcd.)%; C: 65.60 (65.20), H: 2.91 (2.89).

6.1.3. 3-(3′-Chloro-4′-fluorophenyl)isocoumarin (3c)

Yield 73%; m.p. 178–179 °C; IR (KBr) ν_{max} in cm^{-1}: 1716 (C=O), 1114 (C–F) 1059 (C–Cl); ^{1}H NMR ($CDCl_3$, δ-values): 6.92 (1H, s, H-4), 7.22–7.27 (1H, m, H-5′), 7.51–7.57 (2H, m, H-2′,6′), 7.73–7.79 (2H, m, H-5,7), 7.95 (1H, dt, *J* = 7.5, 1.2 Hz, H-6), 8.32 (1H, dd, *J* = 7.8, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 102.0 (C-4), 114.4 (d, *J* = 23.4 Hz, C-5′), 126.1 (C-5), 135.1 (C-6), 126.5 (C-7), 127.6 (d, *J* = 3.0 Hz C-1′), 128.6 (d, *J* = 8.1 Hz, C-2′), 129.2(C-8a), 129.7 (C-8), 133.1 (d, *J* = 8.1 Hz, C-6′), 135.1 (d, *J* = 23.4 Hz, C-3′), 137.0 (C-4a), 151.3 (C-3), 157.2 (C-1), 161.8 (d, *J* = 250.2 Hz, C-4′) ppm; MS (EI, 70 eV): *m*/*z* (%); =274 ($M^{+\bullet}$, 84), 246 (94.0), 227 (2.0), 211 (100), 157 (20.5), 145 (3.5), 117 (5.6), 89 (40.0); Elemental Analysis: Found (Calcd.)%; C: 65.60 (64.99), H: 2.91 (2.95).

6.1.4. 3-(4′-Chloro-2′-fluorophenyl)isocoumarin (3d)

Yield 63%; m.p. 137–138 °C; IR (KBr) ν_{max} in cm^{-1}: 1726(C=O), 1158 (C–F), 1061 (C–Cl); ^{1}H NMR ($CDCl_3$, δ-values): 7.20 (1H, s, H-4), 7.22–7.30 (2H, m, H-3′,5′), 7.52–7.58 (2H, m, H-5,6′), 7.63 (1H, dt, *J* = 7.5, 1.2 Hz, H-7), 7.98 (1H, dt, *J* = 8.4, 1.2 Hz, H-6), 8.33 (1H, dd, *J* = 7.5, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 107.3 (d, *J* = 2.4 Hz, C-4), 117.2 (d, *J* = 21.9 Hz, C-3′), 118.8 (d, *J* = 21.9 Hz, C-1′), 125.1 (d, *J* = 8.1 Hz, C-6′), 125.8 (C-5), 126.5 (C-7), 128.7 (C-8a), 129.1 (d, *J* = 3.0 Hz, C-5′), 129.6 (C-8), 135.0 (C-6), 136.2 (d, *J* = 8.1 Hz, C-4′), 137.2 (C-4a), 147.2 (d, *J* = 7.3 Hz, C-3), 157.9 (C-1), 161.6 (d, *J* = 251 Hz, C-2′) ppm; MS (EI, 70 eV): *m*/*z* (%); =274 ($M^{+\bullet}$, 92), 246 (85.5), 227 (1.2), 211 (100), 157 (32.3), 145 (1.1), 117 (3.2), 89 (57.4); Elemental Analysis: Found (Calcd.)%; C: 65.60 (65.84), H: 2.91 (2.84).

6.2. General synthetic procedure for 2-{2-(dihalophenyl)-2′-oxoethyl}benzoic acids 4(a–d)

Solution of 3-(dihalophenyl)isocoumarins (1.0 g, 3.6 mmol) **4(a–d)** in ethanol (25 mL) and potassium hydroxide (5%, 30 mL) was refluxed for 5 h. After cooling, the reaction mixture was evaporated under reduced pressure to remove ethanol. Cold water (20 mL) was then added and the reaction mixture was acidified with dilute hydrochloric acid and extracted with dichloromethane (2× 25 mL). The solvent was rotary evaporated to afford crude solid which was recrystallized from ethyl acetate to give 2-{2-(Dihalophenyl)-2′-oxoethyl}benzoic acid **4(a–d)**.

6.2.1. 2-{2-(2′′-chloro-4′′-fluorophenyl)-2′-oxoethyl}benzoic acids (4a)

Yield 78%; m.p. 168–169 °C; **IR** (KBr) ν_{max} in cm^{-1}: 3115–2680 (O–H), 1722 (C=O), 1689 (acidic C=O); ^{1}H NMR ($CDCl_3$, δ-values):

4.70 (2H, s, H-1′), 7.05–7.12 (1H, m, H-5″), 7.23–7.27 (1H, m, H-3″), 7.33 (1H, dd, *J* = 7.5, 1.2 Hz, H-3), 7.42 (1H, dt, *J* = 7.8, 1.2 Hz, H-5), 7.58 (1H, dt, *J* = 7.5, 1.5 Hz, H-4), 7.66–7.74 (1H, m, H-6″), 8.16 (1H, dd, *J* = 7.5, 1.5 Hz, H-6), 11.62 (1H, s, acidic-H) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 37.2 (C-1′), 112.5 (d, *J* = 22.5 Hz, C-5″), 116.7 (d, *J* = 22.8 Hz, C-3″), 127.6 (C-5), 128.8 (C-6), 129.6 (C-3), 130.5 (C-4), 130.8 (d, *J* = 9.0 Hz, C-6″), 130.9 (C-1), 133.0 (d, *J* = 3.0 Hz, C-1″), 134.2 (d, *J* = 9.0 Hz, C-2″), 138.0 (C-2), 162.7 (d, *J* = 252 Hz, C-4″), 168.4 (C-acidic), 201.9 (C-2′) ppm; MS (EI, 70 eV): *m*/*z* (%); =292 ($M^{+\bullet}$, 2.2), 274 (92), 246 (100), 211 (74.6), 163 (6), 157 (34.3), 135 (1.4), 129 (26.2), 117 (3.2), 109 (11.2), 93 (10.4), 91(28.8); Elemental Analysis: Found (Calcd.)%; C: 61.54 (61.03), H: 3.42 (3.40).

6.2.2. 2-{2-(2″-Chloro-6″-fluorophenyl)-2′-oxoethyl}benzoic acid (4b)

Yield 82%; m.p. 157–158 °C; IR (KBr) ν_{max} in cm^{-1}: 3072–2654 (O–H), 1719 (C=O), 1681 (acidic C=O); ^{1}H NMR ($CDCl_3$, δ-values): 4.71 (2H, s, H-1′), 7.06 (1H, dt, *J* = 8.1, 0.9 Hz, H-4″), 7.23 (1H, d, *J* = 7.8 Hz, H-3″), 7.29–7.38 (2H, m, H-3,5″), 7.45 (1H, dt, *J* = 7.5, 1.2 Hz, H-5), 7.59 (1H, dt, *J* = 7.5, 1.5 Hz, H-4), 8.18 (1H, dd, *J* = 8.1, 1.5 Hz, H-6), 11.77 (1H, s, acidic-H) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 37.5 (d, *J* = 2.2 Hz, C-1′), 113.5 (d, *J* = 21.9 Hz, C-5″), 123.4 (d, *J* = 2.9 Hz, C-3″), 124.8 (d, *J* = 21.9 Hz, C-1″), 127.8 (C-5), 128.6 (C-3), 130.0 (C-6), 130.8 (C-2), 133.9 (C-4), 134.4 (d, *J* = 8.4 Hz, C-2″), 135.2 (d, *J* = 8.4 Hz, C-4″), 138.5 (C-1), 160.5 (d, *J* = 249 Hz, C-6″), 169.2 (C-acidic-C), 200.7 (d, *J* = 7.1 Hz, C-2′) ppm; MS (EI, 70 eV): *m*/*z* (%); =292 ($M^{+\bullet}$, 1.7), 274 (89.5), 246 (100), 211 (67.8), 163 (7.2), 157 (39.6), 135 (2.7), 129 (34.2), 117 (5.1), 109 (9.3), 93 (8.9), 91(21.7); Elemental Analysis: Found (Calcd.)%; C: 61.54 (60.98), H: 3.42 (3.38).

6.2.3. 2-{2-(3″-chloro-4″-fluorophenyl)-2′-oxoethyl}benzoic acid (4c)

Yield 80%; m.p. 259–260 °C. IR (KBr) ν_{max} in cm^{-1}: 3095–2634 (O–H), 1728 (C=O), 1686 (acidic C=O); ^{1}H NMR ($CDCl_3$, δ-values): 4.73 (2H, s, H-1′), 7.08–7.19 (1H, m, H-5″), 7.33 (1H, d, *J* = 7.5 Hz, H-3), 7.41 (1H, dt, *J* = 7.8, 1.2 Hz, H-5), 7.54 (1H, dt, *J* = 7.5, 1.5 Hz, H-4), 7.64–7.72 (1H, m, H-6″), 7.78–7.87 (1H, m, H-2″), 8.11 (1H, dd, *J* = 7.5, 1.2 Hz, H-6) 11.72 (1H, s, acidic-H) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 36.9 (C-1′), 115.7 (d, *J* = 23.4 Hz, C-5″), 119.6 (d, *J* = 23.4 Hz, C-3″), 126.9 (C-5), 127.5 (d, *J* = 8.1 Hz, C-6″), 128.5 (d, *J* = 8.1 Hz, C-2″), 129.7 (C-3), 129.9 (C-6), 130.2 (C-2), 132.8 (d, *J* = 3.0 Hz, C-1″), 133.8 (C-4), 137.4 (C-1), 163.9 (d, *J* = 250.2 Hz, C-4″), 168.9 (C-acidic), 201.5 (C-2′) ppm; MS (EI, 70 eV): *m*/*z* (%); =292 ($M^{+\bullet}$, 2.1), 274 (82), 246 (100), 211 (70.7), 163 (9.1), 157 (47.3), 135 (1.9), 129 (31.7), 117 (2.8), 109 (12.2), 93 (13.1), 91(32.1); Elemental Analysis: Found (Calcd.)%; C: 61.54 (61.45), H: 3.42 (3.43).

6.2.4. 2-{2-(4″-Chloro-2″-fluorophenyl)-2′-oxoethyl}benzoic acids (4d)

Yield 82%; m.p. 184–186 °C; IR (KBr) ν_{max} in cm^{-1}: 3185–26540 (O–H), 1734 (C=O), 1690 (acidic C=O); ^{1}H NMR ($CDCl_3$, δ-values): 4.69 (2H, s, H-1′), 7.01 (1H, dd, *J* = 8.1, 1.8 Hz, H-3″), 7.21–7.30 (2H, m, H-3, 5″), 7.43 (1H, dt, *J* = 7.5, 1.2 Hz, H-5), 7.56 (1H, dt, *J* = 7.5, 1.5 Hz, H-4), 7.79–7.88 (1H, m, H-6″), 8.14(1H, dd, *J* = 8.1, 1.5 Hz, H-6), 11.70 (1H, s, acidic-H) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 38.9 (d, *J* = 2.3 Hz, C-1′), 117.5 (d, *J* = 21.9 Hz, C-3″), 124.5 (d, *J* = 21.9 Hz, C-1″), 125.4 (d, *J* = 3.0 Hz C-5″), 126.8 (C-6), 127.5 (C-4), 129.6 (C-8), 130.5 (C-8a), 131.8 (d, *J* = 8.1 Hz, C-6″), 133.4 (C-4), 137.1 (C-2), 138.4 (d, *J* = 8.1 Hz, C-4″), 158.4 (d, *J* = 251 Hz, C-2″), 167.4 (C-acidic), 198.1 (d, *J* = 6.9 Hz, C-2′) ppm; MS (EI, 70 eV): *m*/*z* (%); =292 ($M^{+\bullet}$, 1.3), 274 (87.6), 246 (100), 211 (77.6), 163 (9.2), 157 (44.1), 135 (4.1), 129 (28.3), 117 (4.2), 109 (10.2), 93 (12.1), 91(29.5); Elemental Analysis: Found (Calcd.)%; C: 61.54 (61.13), H: 3.42 (3.57).

6.3. General synthetic procedure for (dl)-3-(dihalophenyl)-3,4-dihydroisocoumarins 6(a–d)

Keto-acids **4(a–d)** (60 mg, 0.2 mmol) were stirred over night at room temperature with sodium borohydride (0.1 g) in sodium hydroxide (1%, 20 mL). The reaction mixture was chilled and acidified with dilute hydrochloric acid to yield hydroxy acids **5(a–d)** which were cyclodehydrated by refluxing with acetic anhydride for 1 h. The reaction mixture was diluted with chilled water (20 mL) and extracted with dichloromethane. The solvent was evaporated under reduced pressure to afford (*dl*)-3-(dihalophenyl)-3,4-dihydroisocoumarins **6(a–d)**.

6.3.1. (dl)-3-(2′-Chloro-4′-fluorophenyl)-3,4-dihydroisocoumarin (6a)

Yield 75%; m.p. 136–138 °C. IR (KBr) ν_{max} in cm^{-1}: 1727 (C=O), 1112 (C–F), 1062 (C–Cl); ^{1}H NMR ($CDCl_3$, δ-values): 3.18 (1H, dd, *J* = 10.8, 2.1 Hz, H-4ii), 3.27 (1H, dd, *J* = 14.1, 7.5 Hz, H-4i), 5.91 (1H, dd, *J* = 14.4, 3.9 Hz, H-3), 6.89–6.95(1H, m, H-3′), 7.06–7.20 (2H, m, H-5′,6′), 7.32 (1H, dd, *J* = 7.8, 3.0 Hz, H-5), 7.47 (1H, dt, *J* = 7.8, 1.2 Hz, H-7), 7.60 (1H, dt, *J* = 7.5, 1.5 Hz, H-6), 8.19 (1H, d, *J* = 7.5 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 35.8 (C-4), 70.6 (C-3), 112.5 (d, *J* = 22.5 Hz, C-5′), 117.5 (d, *J* = 22.5 Hz, C-3′), 124.8 (C-7), 126.8 (C-5), 127.7 (C-8), 128.8 (C-8a), 128.9 (d, *J* = 9.0 Hz, C-6′), 132.1 (C-6), 132.8 (d, *J* = 3.0 Hz, C-1′), 133.1 (d, *J* = 9.0 Hz, C-2′), 140.9 (C-4a) 161.7 (d, *J* = 252 Hz, C-4′), 165.2 (C-1) ppm; MS (EI, 70 eV): *m*/*z* (%); =276 ($M^{+\bullet}$, 5.60), 147 (1.20), 129 (1.9), 119 (10.4), 118 (100), 90 (31.8), 89 (12.2); Elemental Analysis: Found (Calcd.)%; C: 65.09 (65.00), H: 3.62 (3.60).

6.3.2. (dl)-3-(2′-Chloro-6′-fluorophenyl)-3,4-dihydroisocoumarin (6b)

Yield 82%; m.p. 158–159 °C. IR (KBr) ν_{max} in cm^{-1}: 1719 (C=O), 1179 (C–F), 1043 (C–Cl); ^{1}H NMR ($CDCl_3$, δ-values): 3.01 (1H, dd, *J* = 16.5, 3.3 Hz, H-4ii), 3.48 (1H, dd, *J* = 15.9, 13.5 Hz, H-4i), 6.15 (1H, dd, *J* = 12.9, 3.3 Hz, H-3), 7.10 (1H, dt, *J* = 8.1, 3.0 Hz, H-4′), 7.24–7.37 (3H, m, H-3′, 5,5′), 7.47 (1H, dt, *J* = 7.5, 1.2 Hz, H-7), 7.61 (1H, dt, *J* = 7.5, 1.5 Hz, H-6), 8.19 (1H, dd, *J* = 7.8, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 36.0 (d, *J* = 2.4 Hz, C-4), 61.1 (d, *J* = 7.3 Hz, C-3), 113.0 (d, *J* = 21.5 Hz, C-5′), 124.9 (C-7), 125.0 (d, *J* = 3.2 Hz, C-3′), 127.2 (C-5), 128.1 (d, *J* = 21.5 Hz, C-1′), 129.2 (C-8), 130.2 (d, *J* = 8.0 Hz, C-4′), 131.9 (C-6), 133.0 (C-8a), 137.3 (d, *J* = 8.0 Hz, C-2′), 141.2 (C-4a), 160.9 (d, *J* = 249 Hz, C-6′), 164.9 (C-1) ppm; MS (EI, 70ev): *m*/*z* (%); =276 ($M^{+\bullet}$, 7.56), 147 (4.65), 129 (4.6), 119 (12.5), 118 (100), 90 (34.0), 89 (10.9); Elemental Analysis: Found (Calcd.)%; C: 65.09 (65.12), H: 3.62 (3.59).

6.3.3. (dl)-3-(3′-Chloro-4′-fluorophenyl)-3,4-dihydroisocoumarin (6c)

Yield 77%; m.p. 129–130 °C. IR (KBr) ν_{max} in cm^{-1}: 1717 (C=O), 1110 (C–F), 1057 (C–Cl); ^{1}H NMR ($CDCl_3$, δ-values): 3.11 (1H, dd, *J* = 16.5, 3.0 Hz, H-4ii), 3.35 (1H, dd, *J* = 16.2, 12.0 Hz, H-4i), 5.48 (1H, dd, *J* = 12.0, 3.0 Hz, H-3), 6.96 (1H, dd, *J* = 8.7, 3.0 Hz, H-6′), 7.28–7.35 (2H, m, H-5,5′), 7.45 (1H, dt, *J* = 7.8, 1.5 Hz, H-7), 7.51 (1H, d, *J* = 2.1 Hz, H-2′), 7.60 (1H, dt, *J* = 7.5, 1.2 Hz, H-6), 8.16 (1H, dd, *J* = 7.8, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 35.7 (C-4), 78.8 (C-3), 116.9 (d, *J* = 23.7 Hz, C-5′), 120.8 (d, *J* = 3.1 Hz, C-1′), 125.2 (C-7), 126.3 (C-5), 127.0 (d, *J* = 7.7 Hz, C-6′), 128.1 (d, *J* = 7.7 Hz, C-2′), 128.4 (C-8), 128.9 (C-8a), 133.0 (C-6), 136.9 (d, *J* = 23.7 Hz, C-3′), 140.5 (C-4a) 160.5 (d, *J* = 250.2 Hz, C-4′), 165.8 (C-1) ppm; MS (EI, 70 eV): *m*/*z* (%); =276 ($M^{+\bullet}$, 8.76), 147 (2.40), 129 (3.5), 119 (9.65), 118 (100), 90 (37.0), 89 (14.5); Elemental Analysis: Found (Calcd.)%; C: 65.09 (65.14), H: 3.62 (3.63).

6.3.4. (dl)-3-(4′-Chloro-2′-fluorophenyl)-3,4-dihydroisocoumarin (6d)

Yield 78%; m.p. 151–153 °C. IR (KBr) ν_{max} in cm^{-1}: 1731 (C=O), 1117 (C–F), 1080 (C–Cl); ^1H NMR ($CDCl_3$, δ-values): 3.17 (1H, dd, *J* = 10.5, 4.5 Hz, H-4ii), 3.29 (1H, dd, *J* = 16.5, 11.4 Hz, H-4i), 5.83 (1H, dd, *J* = 11.7, 3.6 Hz, H-3), 7.15 (1H, dd, *J* = 9.9, 1.8 Hz, H-3′), 7.23–7.34 (2H, m, H-5′,6′), 7.46 (1H, dt, *J* = 6.3, 1.2 Hz, H-7), 7.52–64 (2H, m, H-5,6), 8.17 (1H, dd, *J* = 7.8, 1.5 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 34.5 (d, *J* = 2.3 Hz, C-4), 73.7 (d, *J* = 7.3 Hz, C-3), 116.4 (d, *J* = 21.9 Hz, C-3′), 120.7 (d, *J* = 21.8 Hz, C-1′), 124.7 (d, *J* = 2.7 Hz, C-5′), 125.8 (C-7), 127.3 (C-5), 128.1 (C-8a), 128.5 (C-8), 132.8 (d, *J* = 8.1 Hz, C-6′), 133.9 (C-6), 135.2 (d, *J* = 8.1 Hz, C-4′), 139.3 (C-4a), 160.7 (d, *J* = 251 Hz, C-2′), 165.2 (C-1) ppm; MS (EI, 70 eV): *m*/*z* (%); =276 ($M^{+\bullet}$, 2.60), 147 (1.30), 129 (3.2), 119 (10.0), 118 (100), 90 (43.1), 89 (18.2); Elemental Analysis: Found (Calcd.)%; C: 65.09 (64.92), H: 3.62 (3.72).

6.4. General synthetic procedure for 3-(dihalophenyl)thioisocoumarins 7(a–d)

Thioisocoumarins **7(a–d)** were synthesized by thionation of isocoumarins **3(a–d)**. Thionation was carried out by refluxing isocoumarins **3(a–d)** (0.5 g, 1.82 mmol) with Lawesson's Reagent [23] (0.89 g, 2.2 mmol) in dry toluene for four hours. Pure thioisocoumarins were obtained from reaction mixture by recrystallization with methanol.

6.4.1. 3-(2′-Chloro-4′-fluorophenyl)thioisocoumarin (7a)

Yield 82%; m.p. 197–198 °C. IR (KBr) ν_{max} in cm^{-1}: 1107 (C–F), 1081(C=S), 1057 (C–Cl); ^1H NMR ($CDCl_3$, δ-values): 7.23–7.27 (1H, m, H-5′), 7.32 (1H, ddd, *J* = 8.7, 2.1, 0.6 Hz, H-3′), 7.37(1H, s, H-4), 7.50–7.58 (2H, m, H-5,6′), 7.76 (1H, dt, *J* = 7.5, 1.2 Hz, H-7), 8.09 (1H, dt, *J* = 8.4, 1.5 Hz, H-6), 8.74 (1H, dd, *J* = 8.1, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 90.0 (C-4), 114.8 (d, *J* = 22.5 Hz, C-5′), 118.2 (d, *J* = 22.5 Hz, C-3′), 125.4 (C-5), 128.5 (d, *J* = 3.3 Hz, C-1′), 129.5 (C-7), 130.0 (d, *J* = 9.0 Hz, C-6′), 130.9 (C-6), 131.4 (C-8), 133.7 (d, *J* = 9.0 Hz, C-2′), 136.8 (C-4a), 140.0 (C-8a), 160.8 (C-3), 164.5 (d, *J* = 252 Hz, C-4′), 200.6 (C-1) ppm; MS (EI, 70 eV): *m*/*z* (%); =290 ($M^{+\bullet}$, 100), 246 (5.7), 161 (4.7), 157(19.8), 133(9.45), 129(18.9), 89(37.4); Elemental Analysis: Found (Calcd.)%; C: 61.96 (61.58), H: 2.75 (2.59), S: 11.02 (10.96).

6.4.2. 3-(2′-Chloro-6′-fluorophenyl)thioisocoumarin (7b)

Yield 84%; m.p. 270–271 °C. IR (KBr) ν_{max} in cm^{-1}: 1162 (C–F), 1085 (C=S), 1061 (C–Cl); ^1H NMR ($CDCl_3$, δ-values): 6.90 (1H, s, H-4), 7.15 (1H, dt, *J* = 8.1, 3.0 Hz, H-4′), 7.32–7.36 (1H, m, H-3′), 7.39–7.46 (1H, m, H-5′), 7.51 (1H, dd, *J* = 7.5, 1.2 Hz, H-5), 7.60 (1H, dt, *J* = 8.1, 1.2 Hz, H-7), 7.78 (1H, dt, *J* = 7.5, 1.2 Hz, H-6), 8.78 (1H, dd, *J* = 8.4, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 90.5 (d, *J* = 2.2 Hz, C-4), 113.8 (d, *J* = 21.7 Hz, C-5′), 123.5 (d, *J* = 21.7 Hz, C-1′), 125.0 (d, *J* = 3.0 Hz, C-3′), 125.8 (C-5), 128.0 (C-7), 129.5 (d, *J* = 8.7 Hz, C-4′), 130.0 (C-8), 130.7 (C-6), 135.1 (d, *J* = 8.7 Hz, C-2′), 140.1 (C-4a), 149.2 (C-8a), 159.4 (d, *J* = 7.2 Hz, C-3), 162.6 (d, *J* = 249 Hz, C-6′), 201.1 (C-1) ppm; MS (EI, 70 eV): *m*/*z* (%); =290 ($M^{+\bullet}$, 100), 246 (3.5), 161 (2.5), 157 (22.4), 133 (10.2), 129 (20.0), 89 (35.9); Elemental Analysis: Found (Calcd.)%; C: 61.96 (61.71), H: 2.75 (2.63), S: 11.02(10.98).

6.4.3. 3-(3′-Chloro-4′-fluorophenyl)thioisocoumarin (7c)

Yield 83%; m.p. 203–204 °C. IR (KBr) ν_{max} in cm^{-1}: 1123 (C–F), 1076 (C=S) 1047 (C–Cl); ^1H NMR ($CDCl_3$, δ-values): 7.08 (1H, s, H-4), 7.23–7.29 (1H, m, H-5′), 7.48–7.55 (2H, m, H-2′,6′), 7.72–7.84 (2H, m, H-5,7), 7.98 (1H, dt, *J* = 7.8, 1.2 Hz, H-6), 8.71 (1H, dd, *J* = 8.1, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 89.8 (C-4), 118.7 (d, *J* = 23.6 Hz, C-5′), 121.7 (d, *J* = 23.6 Hz, C-3′), 126.0 (C-5), 127.1 (d, *J* = 3.0 Hz, C-1′), 127.5 (d, *J* = 8.3 Hz, C-6′), 128.0 (d, *J* = 8.1 Hz, C-2′), 128.8 (C-7), 130.9 (C-8), 135.5 (C-6), 136.7 (C-4a), 144.3 (C-8a), 157.5 (C-3), 162.9 (d, *J* = 250.2 Hz, C-4′), 199.8 (C-1) ppm; MS (EI, 70 eV): *m*/*z* (%); =290 ($M^{+\bullet}$, 100), 246 (5.9), 161 (3.5), 157 (18.5), 133 (8.63), 129 (25.5), 89 (40.0); Elemental Analysis: Found (Calcd.)%; C: 61.96 (61.49), H: 2.75 (2.68), S: 11.02 (10.84).

6.4.4. 3-(4′-Chloro-2′-fluorophenyl)thioisocoumarin (7d)

Yield 77%; m.p. 190–192 °C. IR (KBr) ν_{max} in cm^{-1}: 1171 (C–F), 1109(C=S), 1072 (C–Cl); ^1H NMR ($CDCl_3$, δ-values): 7.23–7.28 (1H, m, H-3′), 7.32 (1H, dd, *J* = 8.7, 2.1 Hz, H-5′), 7.38 (1H, s, H-4), 7.51–7.58 (2H, m, H-5, 6′), 7.77 (1H, dt, *J* = 7.8, 1.2 Hz, H-7), 8.09 (1H, dt, *J* = 7.5, 1.2 Hz, H-6), 8.74 (1H, dd, *J* = 8.1, 1.2 Hz, H-8) ppm; ^{13}C NMR ($CDCl_3$, δ-values): 92.4 (d, *J* = 2.1 Hz, C-4), 117.1 (d, *J* = 2.9 Hz, C-5′), 118.3 (d, *J* = 22.2 Hz, C-3′), 125.3 (C-5), 126.9 (d, *J* = 22.3 Hz, C-1′), 129.4 (C-7), 129.9 (d, *J* = 8.2 Hz, C-6′), 131.5 (C-6), 132.7 (C-8), 136.5 (d, *J* = 8.2 Hz, C-4′), 137.8 (C-4a), 140.0 (C-8a), 157.9 (d, *J* = 7.1 Hz, C-3), 161.3 (d, *J* = 252 Hz, C-2′), 199.9 (C-1) ppm; MS (EI, 70 eV): *m*/*z* (%); =290 ($M^{+\bullet}$, 100), 246 (4.7), 161 (3.9), 157 (25.5), 133 (11.7), 129 (20.5), 89 (48.4); Elemental Analysis: Found (Calcd.)%; C: 61.96 (61.64), H: 2.75 (2.61), S: 11.02 (10.82).

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