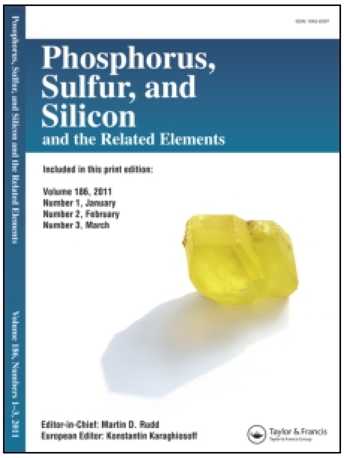

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Synthesis and Antiproliferative Activity of Some 1H-Isochromen-1-ones and Their Thio Analogues

P. Manivel[ab]; Ashok Sharma[c]; T. Maiyalagan[a]; M. R. Rajeswari[c]; F. Nawaz Khan[a]
[a] Chemistry Division, School of Science and Humanities, VIT University, Vellore, Tamil Nadu, India [b] Syngene International Limited, Bangalore, Karnataka, India [c] Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India

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SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF SOME 1H-ISOCHROMEN-1-ONES AND THEIR THIO ANALOGUES

P. Manivel,[1,3] Ashok Sharma,[2] T. Maiyalagan,[1]
M. R. Rajeswari,[2] and F. Nawaz Khan[1]
[1]Chemistry Division, School of Science and Humanities, VIT University, Vellore, Tamil Nadu, India
[2]Department of Biochemistry, All India Institute of Medical Sciences, Ansari Nagar, New Delhi, India
[3]Syngene International Limited, Bangalore, Karnataka, India

A series of 1H-isochromen-1-one derivatives and their thio analogues were synthesized by thionation using Lawesson's reagent for evaluation of their cytotoxic effect on cancer cells. The effect of these derivatives was tested on the growth of human epidermoid carcinoma A431 cell line by MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] colorimetric assay. MTT assay revealed that these new compounds are potent inhibitors of cell growth.

Supplemental materials are available for this article. Go to the publisher's online edition of* Phosphorus, Sulfur, and Silicon and the Related Elements *to view the free supplemental file.

Keywords Antiproliferative agents; cell growth; 1H-isochromen-1-one; 1H-isochromen-1-thione

INTRODUCTION

It is well known that skin is the body's largest organ, and serves as a protective barrier against the deleterious effects of environmental hazards.[1] However, skin cancer is more common in the U.S.,[2] with males having a 1:7 and females having a 1:5 lifetime chance of developing skin cancer.[3] Skin cancer incidence is approximately 120 in 100,000 in India per year.[4, 5] The two major types of skin cancers are non-melanoma and melanoma. The incidence of both non-melanoma and melanoma skin cancers has been rising at an alarming rate for the past several years. Basal cell carcinoma and squamous cell carcinomas are the most common non-melanoma skin cancers (NMSC). The NMSCs are prevalent among Caucasians. Recent epidemiology suggests that individuals with a history of non-melanoma skin cancer have risk of melanoma. Chemoprevention is a strategy to use natural or synthetic

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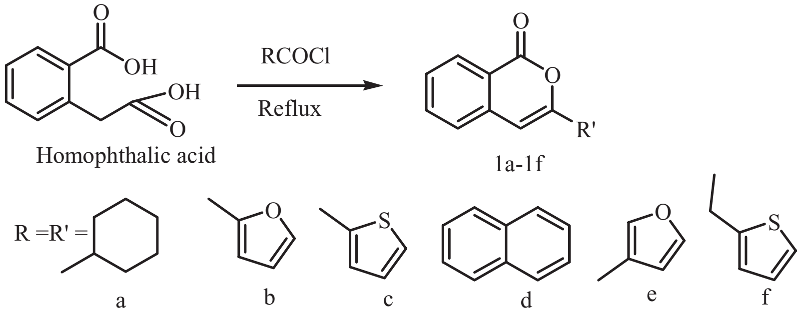
Address correspondence to F. Nawaz Khan, Chemistry Division, School of Science and Humanities, VIT University, Vellore, Tamil Nadu 632 014, India. E-mail: nawaz_f@yahoo.co.in

substances to prevent the initiation, and/or promotion, and/or progression of events during cancer development.[6] Recent studies have shown that several marine natural products produce promising biological activity, specifically as chemopreventive activity against tumor development including skin cancer.[7–14]

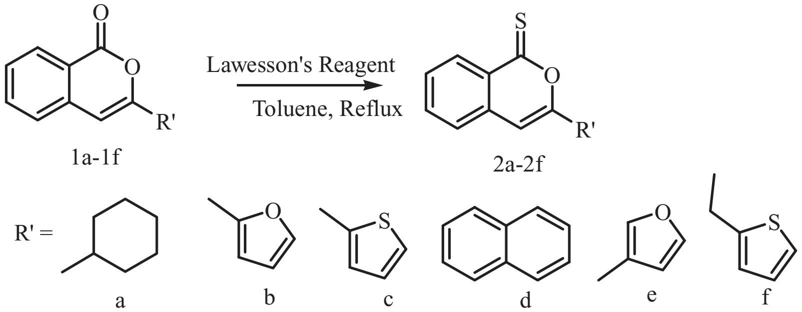
1H-isochromen-1-ones and their analogues are widely distributed in nature and constitute a class of compounds with a broad spectrum of pharmacological properties, so in continuation of our research,[15–18] we decided to synthesize new isocoumarins and their thioanalogues.[18–23] The antiproliferative activity of such compounds was assessed on the human epidermoid carcinoma cell line A431, by MTT-colorimetric assay following the reported procedure.[24,25]

RESULTS AND DISCUSSION

Schemes 1 and 2 show the synthetic route to the target compounds **1a–1f** and **2a–2f**. Homophthalic acid was reacted with various acid chlorides such as cyclohexanecarbonyl chloride, naphthalenecarbonylchloride, furan-2-carbonyl chloride, and thiophene-2-carbonyl chloride in the absence of any solvent at 200°C for 4–5 h to give the corresponding 1H-isochromen-1-one derivatives **1a–1f** (Scheme 1). In Scheme 2, it is shown that 1H-isochromen-1-thiones **2a–2f** were prepared from the corresponding 1H-isochromen-1-ones by treatment with Lawesson's reagent in toluene at reflux temperatures. The results are presented in Table I.



Scheme 1 Synthesis of isocoumarins.



Scheme 2 Synthesis of thioisocoumarins.

Table I Synthesis of different 3-substituted isocoumarins, **1a–1f**, from homophthalic acid[a] and 3-substituted thioisocoumarins, **2a–2f**, from isocoumarins using Lawesson's reagent[c]

S. No.	Acid chloride, **RCOCl**	Isocoumarin, **1**/ Thioisocoumarin, **2** **R**′	Yield,[b] **1** %	Yield,[d] **2** %
1	Cyclohexanecarbonyl chloride	Cyclo C_6H_{11}, **1a**	86	87
2	Furan-2-carbonyl chloride	2-Furyl, **1b**	90	89
3	Thiophene-2-carbonyl chloride	2-Thiophenyl, **1c**	89	90
4	1-Naphthoyl chloride	1-Naphthyl, **1d**	85	81
5	Furan-3-carbonyl chloride	3-Furyl, **1e**	88	92
6	2-(Thiophen-2-yl)acetyl chloride	(Thiophen-2-yl)methyl), **1f**	85	83

[a] Homophthalic acid (20mmol), acid chloride (80 mmol).
[b] Isolated yield.
[c] Isocoumarins (1mmol), Lawesson reagent (1.2 mmol).
[d] Isolated yield.

Taking into consideration the structure of 1H-isochromen-1-ones and 1H-isochromen-1-thiones containing β-unsaturated carbonyl group as a Michael acceptor, an active moiety often employed in the design of anticancer drugs, we prepared some novel 1H-isochromen-1-ones and their thioanalogues (Schemes 1 and 2) in order to evaluate their cytotoxicity against human epidermoid carcinoma cells A431.

The synthesized 1H-isochromen-1-ones and thioderivatives were tested for their ability to inhibit tumor-promoting activity. Cell viability was monitored by the MTT assay. IC_{50} values were calculated from curves constructed by plotting suppression ratio (%) vs. compound concentration. Each assay, as well as the whole experiment, was performed at least three times. Compounds **1a–d** and **2a–d** were evaluated for cytotoxicity in vitro against human epidermoid carcinoma A431 cells. The 50% growth inhibition concentrations (GI_{50}) of these compounds are summarized in Table II and represented by graphs shown in Figures 1 and 2 (Table II and Figures 1 and 2 are available online in the Supplemental Materials). Thus, compounds **1a–d** and **2a–d** exhibited significant antitumor activity with GI_{50} values in range of 15 ng to 21 μg/mL. The GI_{50} values of the test compounds were determined by using the MTT antiproliferative assay as described in the methods; values are mean ± standard deviations (s.d) of triplicate determination. (See the Supplemental Materials online, Table II, Figures 1 and 2.)

Overall, the present results on antiproliferation studies suggest that 1H-isochromen-1-ones and their thioanalogues could be effective antiproliferative agents for skin cancer. This chemopreventive effect may involve multiple mechanisms including induction of apoptosis and decrease in cell proliferation. Pretreatment with 1H-isochromen-1-ones and thioanalogues decreased tumor development at a very low concentration (nmoles per application). It is possible that it may provide complete protection at relatively higher concentrations. Future investigations involving concentration–response against both chemically and UVB-induced skin tumor, and mechanistic studies are needed to fully evaluate the chemopreventive effects of 1H-isochromen-1-ones and their thioanalogues.

EXPERIMENTAL

The materials were purchased from Sigma–Aldrich and Merck, and were used without any additional purification. All reactions were monitored by thin layer chromatography (TLC) on gel F254 plates. The silica gel (230–400 meshes) for column chromatography was purchased from Spectrochem Pvt. Ltd., India. IR spectra were recorded on a Nucon Infrared spectrophotometer using KBr pellets. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker 400 MHz spectrometer in $CDCl_3$ or DMSO (with TMS for ^{1}H and DMSO for ^{13}C NMR as internal references). LC–MS analyses were performed with LCMS-Agilent-1100 series Ion Trap.

General Procedure for the Preparation of 3-Substituted 1H-Isochromen-1-ones, 1a–1f

A mixture of homophthalic acid (20mmol) and acid chloride (80 mmol) was heated under reflux at 200°C in an oil bath for 4–5 h. The completion of the reaction was monitored by TLC, and then the mixture was chromatographed on silica gel using hexane and ethyl acetate (9:1) as eluent. The product 3-substituted 1H-isochromen-1-one was obtained with yields of 85% to 90%. The solid obtained was characterized by LC-MS, ^{1}H NMR, and ^{13}C NMR techniques.

3-Cyclohexyl-1H-isochromen-1-one (1a). Colorless solid, mp, 94–95°C, IR (ν cm^{-1}), 3071, 1738, 1687, 2671, 2554. ^{1}H NMR ($CDCl_3$): δ 8.26–8.24 (d, J = 8 Hz, 1H), 7.69–7.65 (m, 1H), 7.47–7.43 (m, 1H), 7.38–7.36 (d, J = 8 Hz, 1H) (Aromatic protons), 6.24 (s, 1H) (O—C=C*H*), 2.48–2.42 (m, 1H), 2.06–2.03 (m, 2H), 1.88–1.84 (m, 2H), 1.77–1.73 (m, 1H), 1.51–1.39 (m, 5H) (Alicyclic protons). ^{13}C NMR ($CDCl_3$): δ 163.15 (*C*=O), 137.76, 134.63, 129.45, 127.48, 125.23, 120.28 (Aromatic carbons), 100.88 (O—C=*C*), 41.68, 30.58, 2 × 25.94, 2 × 25.88 (Alicyclic carbons), LCMS-229.3, $C_{15}H_{16}O_2$, Mol. Wt.: 228.29, Calculated C, 78.92; H, 7.06, found C, 78.84; H, 6.95.

The reaction of homophthalic acid with various acid chlorides gave different 1H-isochromen-1-ones, **1a–1f** (Table I).

3-(Furan-2-yl)-1H-isochromen-1-one (1b). Colorless solid, mp, 121–122°C IR (ν cm^{-1}), 3058, 1727, 1666, 2653, 2547. ^{1}H NMR ($CDCl_3$): δ 8.30–8.28 (d, J = 8 Hz, 1H), 7.73–7.69 (m, 1H), 7.52–7.43 (m, 3H), 6.97–6.96 (d, J = 4 Hz, 1H), 6.93–6.92 (d, J = 4 Hz, 1H) (Aromatic and furan ring protons), 6.54 (s, 1H) (O—C=C*H*). ^{13}C NMR ($CDCl_3$) δ 161.60 (*C*=O), 146.96, 146.12, 144.01, 137.34, 134.96, 129.85, 127.99, 125.96, 120.50, 112.14, 110.14 (Aromatic and heterocycle carbons), 100.04 (O—C=*C*). LCMS-212, $C_{13}H_8O_3$, Mol. Wt.: 212.20, Calculated C, 73.58; H, 3.80, found C, 73.47; H, 3.72.

3-(Thiophen-2-yl)-1H-isochromen-1-one (1c). Pale yellow solid, mp, 101–102°C, IR (ν cm^{-1}), 3055, 1727, 1677, 2667, 2549. ^{1}H NMR ($CDCl_3$): δ 8.31–8.29 (d, J = 8 Hz, 1H), 7.74–7.70 (m, 1H), 7.63–7.62 (m, 1H), 7.51–7.46 (m, 2H), 7.42–7.41 (d, J = 4 Hz, 1H), 7. 14–7. 12 (d, J = 8 Hz, 1H) (Aromatic and thiophene ring protons), 6.81 (s, 1H) (O—C=C*H*). ^{13}C NMR ($CDCl_3$) δ 161.72 (*C*=O), 149.51, 137.48, 135.69, 134.96, 129.86, 128. 13, 127.96, 126.20, 125.71, 120.37 (Aromatic and heterocycle carbons), 100.86 (O—C=*C*). LCMS-228, $C_{13}H_8O_2S$, Mol. Wt.: 228.27, Calculated C, 68.40; H, 3.53; S, 14.05, found C, 68.24; H, 3.33; S, 13.84.

3-(Naphthalen-1-yl)-1H-isochromen-1-one (1d). Colorless solid, mp, 143–145°C IR (ν cm^{-1}), 3073, 1744, 1674, 2661, 2546. ^{1}H NMR ($CDCl_3$): δ 8.41–8.39 (d, J = 8 Hz, 1H), 8.27–8.25 (d, J = 8 Hz, 1H), 7.98–7.92 (m, 2H), 7.81–7.76 (m, 2H), 7.60–7.53 (m, 5H) (Aromatic and naphthalene ring protons), 6.84 (s, 1H) (O—C=C*H*), ^{13}C NMR ($CDCl_3$) δ 162. 66 (*C*=O), 154. 77, 137.48, 136. 94, 133.78, 130. 79, 130.73, 130. 56, 129.73, 128. 6, 128. 44, 127.73, 127.12, 126 38, 125.92, 125.14, 125.06, 120.57, (Aromatic and naphthalene ring carbons) 107.14 (O—C=*C*). LCMS-272.9, $C_{19}H_{12}O_2$, Mol. Wt.: 272.30, Calculated C, 83.81; H, 4.44, found C, 83.68; H, 4.32.

3-(Furan-3-yl)-1H-isochromen-1-one (1e). Colorless solid, mp, 118–120°C, IR (ν cm^{-1}), 3066, 1727, 1674, 2669, 2548. ^{1}H NMR ($CDCl_3$): δ 8.31–8.29 (d, J = 8 Hz, 1H), 7.99 (s, 1H), 7.71–7.69 (m, 1H), 7.51–7.44 (m, 3H), 6.71–6.70 (m, 1H) (Aromatic and furan ring protons), 6.63 (s, 1H) (O—C=C*H*), ^{13}C NMR ($CDCl_3$) δ 162. 03 (*C*=O), 148.77, 144.19, 141.69, 137.50, 134.88, 129.80, 127.85, 125.50, 120.52, 120. 18, 107. 02 (Aromatic and furan ring carbons), 101.23 (O—C=*C*). LCMS-212, $C_{13}H_8O_3$, Mol. Wt.: 212.20, Calculated C, 73.58; H, 3.80, found C, 73.46; H, 3.71.

3-((Thiophen-2-yl) methyl)-1H-isochromen-1-one (1f). Pale yellow solid, mp, 95–97°C, IR (ν cm^{-1}), 3053, 1720, 1664, 2669, 2548. ^{1}H NMR ($CDCl_3$): δ 8.28–8.26 (d, J = 8 Hz, 1H), 7. 71–7. 66, (m, 1H), 7.50–7.46 (m, 1H), 7.37–7.35 (m, 1H), 7.27–7.24 (m, 1H), 7.03–7.00 (m, 2H) (Aromatic and thiophene ring protons), 6.28 (s, 1H) (O—C=C*H*), 4. 07 (s, CH_2, 2H). ^{13}C NMR ($CDCl_3$) δ 162.54 (*C*=O), 155.91, 137. 25, 137.21, 134.76, 129.61, 128. 01, 127.17, 127.01 125.00, 120.23, 103.70 (O—C=*C*), 33.82 (*C*H_2), LCMS-242, $C_{14}H_{10}O_2S$, Mol. Wt.: 242.29, Calculated C, 69.40; H, 4.16; O, 13.21; S, 13.23, found C, 69.3; H, 4.03; S, 13.01.

General Procedure for the Preparation of 3-Substituted Thio 1H-Isochromen-1-ones, 2a–2f

The reaction of the 1H-isochromen-1-ones (1 mmol), **1a–f** with Lawessons' reagent (1.2 mmol) in toluene 20 mL at reflux temperature 110°C results in an O/S-exchange at the carbonyl carbon atom with formation of the thio 1H-isochromen-1-ones **2a–f** in good yield of 81–92%. The reaction proceeds smoothly and is complete within a maximum of 5–6 h. The completion of the reaction was monitored by TLC, and then the mixture chromatographed on silica gel using hexane and ethyl acetate (9:1) as eluent.

3-Cyclohexyl-1H-isochromene-1-thione (2a). Pale yellow solid, mp, 68–70°C, IR (ν cm^{-1}), 3049, 2920, 2848, 1646, 1550, 1473, 1444, 1338, 1283, 1167, 1141, 925, 828, 769, 567 . ^{1}H NMR ($CDCl_3$): δ 8.71–8.69 (d, J = 8 Hz, 1H), 7.72–7.67 (m, 1H), 7.49–7.44 (m, 1H), 7.38–7.36 (m, 1H) (Alicyclic ring protons), 6.49 (s, 1H) (O—C=C*H*), 2.62–2.57 (t, J = 10 Hz 1H), 2.12–2.09 (m, 2H), 1.90–1.86 (m, 2H), 1.79–1.74 (m, 1H), 1.52–1.26 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 201.50 (*C*=S), 165.70, 134.98, 132.72, 132.36, 129.9, 128.54, 125.76, 104.00 (O—C=*C*), 41.68, 30.81, 25.91, 25.80 (Alicyclic ring carbons). LCMS-244.7, $C_{15}H_{16}OS$ Mol.Wt: 244.09, Calculated C, 73.73; H, 6.60; S, 13.12, found C, 73.65; H, 6.44; S, 13.03

3-(Furan-2-yl)-1H-isochromene-1-thione (2b). Pale yellow solid, mp, 112–114°C, IR (ν cm^{-1}), 3137, 3063, 1649, 1602, 1541, 1466, 1335, 1284, 1168, 1149, 986, 815, 739, 589. ^{1}H NMR ($CDCl_3$): δ 8.72–8.70 (d, J = 8 Hz, 1H), 7.74–7.70 (m, 1H), 7.55–7.46 (m, 3H), 7.11–7.06 (m, 1H), 6.98–6.89 (m, 1H) (Aromatic and furan ring protons), 6.57 (s, 1H) (O—C=C*H*), ^{13}C NMR ($CDCl_3$) δ 199.58 (*C*=S), 149.07, 144.32, 135.18, 133.10, 131.79, 128.98, 126.41, 125.97, 112.29, 112.15, 110.90 (Aromatic and furan ring carbons), 102.79 (O—C=*C*). LCMS-228, $C_{13}H_8O_2S$, Mol. Wt.: 228.27, Calculated C, 68.40; H, 3.53; S, 14.05, found C, 68.24; H, 3.39; S, 13.95

3-(Thiophen-2-yl)-1H-isochromene-1-thione (2c). Pale yellow solid, mp, 101–103°C, IR (ν cm^{-1}), 3097, 3055, 2920, 2850, 1629, 1549, 1474, 1420, 1346, 1223, 1165, 1147, 1110, 824, 700. ^{1}H NMR ($CDCl_3$): δ 8.72–8.70 (d, J = 8 Hz, 1H), 7.74–7.70 (m, 2H), 7.63–7.62 (m, 1H), 7.50–7.41 (m, 2H), 7.16–7.12 (m, 1H) (Aromatic and thiophene ring protons), 6.97 (s, 1H) (O—C=C*H*). ^{13}C NMR ($CDCl_3$) δ 199.73 (*C*=S), 135.15, 133.06, 132.05, 130.04, 128. 20, 127.90, 126.79, 126.17 (Aromatic and thiophene ring carbons), 103.61 (O—C=*C*), LCMS-244, $C_{13}H_8OS_2$, Mol. Wt.: 244.33, Calculated C, 63.90; H, 3.30; S, 26.25, found C, 63.74; H, 3.19; S, 26.18

3-(Naphthalen-1-yl)-1H-isochromene-1-thione (2d). Pale yellow solid, mp, 114–115°C, IR (ν cm^{-1}), 3040, 2920, 2849, 1637, 1551, 1506, 1472, 1472, 1343, 1274, 1164, 1110, 1085, 878, 766, 571. ^{1}H NMR ($CDCl_3$): δ 8.83–8.81 (d, J = 8 Hz, 1H), 8.26–8.23 (m, 1H), 8.0–7.91 (m, 2H), 7.82–7.75 (m, 2H), 7.62–7.50 (m, 5H) (Aromatic and naphthalene ring protons), 7.03 (s, 1H) (O—C=C*H*). ^{13}C NMR ($CDCl_3$) δ 200. 84 (*C*=S), 157. 77, 135. 16, 133. 79, 132.88, 130. 92, 130.73, 130. 19, 130. 17, 129.43, 128.66, 128. 03, 127.38, 126.40, 126 38, 125.08, 124.88 (Aromatic and naphthalene ring carbons), 109. 84 (O—C=*C*). LCMS-288, $C_{19}H_{12}OS$, Mol. Wt.: 288.36, Calculated C, 79.14; H, 4.19; S, 11.12, found C, 79.02; H, 4.05; S, 11.01

3-(Furan-3-yl)-1H-isochromene-1-thione (2e). Pale yellow solid, mp, 130–132°C, IR (ν cm^{-1}), 3107, 3065, 2924, 2848, 1659, 1547, 1508, 1485, 1474, 1339, 1285, 1158, 1117, 1084, 821, 791, 589. ^{1}H NMR ($CDCl_3$): δ 8.72–8.70 (d, J = 8 Hz, 1H), 8.09–8.07 (m, 1H), 7.73–7.69 (m, 1H), 7.52–7.42 (m, 3H), 6.81 (s, 1H) (Aromatic and furan ring protons), 6.73 (s, 1H) (O—C=C*H*). ^{13}C NMR ($CDCl_3$) δ 200. 16 (*C*=S), 151.75, 144.34, 142. 11, 135. 11, 133.03, 132. 02, 130. 09, 128.84, 125.97, 119. 66, 107. 00 (Aromatic and furan ring carbons), 104.00 (O—C=*C*). LCMS-228, $C_{13}H_8O_2S$, Mol. Wt.: 228.27, Calculated C, 68.40; H, 3.53; S, 14.05, found C, 68.27; H, 3.42; S, 13.97

3-((Thiophen-2-yl)methyl)-1H-isochromene-1-thione (2f). Pale yellow solid, mp, 111°C, IR (ν cm^{-1}), 3063, 2920, 2856, 1724, 1639, 1547, 1486, 1437, 1336, 1274, 1155, 1119, 938, 847, 781, 573. ^{1}H NMR ($CDCl_3$): δ δ 8.78–8.76 (d, J = 8 Hz, 1H), 8. 09–8. 06, (m, 1H), 7.50–7.46 (m, 1H), 7.37–7.35 (m, 1H), 7.27–7.24 (m, 1H), 7.03–7.00 (m, *CH*$_2$, 2H) (Aromatic and thiophene ring protons), 6.53 (s, 1H) (O—C=C*H*), 4. 07 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 200. 54 (*C*=S), 155.97, 137. 30, 137.31, 134.74, 129.54, 128. 41, 127.37, 127.20 125.17, 120.11 (Aromatic and thiophene ring carbons), 103.64 (O—C=*C*), 33.79 (*C*H$_2$). LCMS-258, $C_{14}H_{10}OS_2$, Mol. Wt.: 258.36, Calculated C, 65.08; H, 3.90; S, 24.82, found C, 64.92; H, 3.74; S, 24.67

Antiproliferative Assay

A431 (human epidermoid carcinoma cells) were obtained from NCCS, Pune, India, and were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Life Technologies, Gaithersburg, MD, USA). Cells were routinely sub-cultured twice per week and maintained in a humidified atmosphere with 5% CO_2 at 37°C. (See the Supplemental Materials online.)

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