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Synthesis of new coumarin 3-(*N*-aryl) sulfonamides and their anticancer activity

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Abstract—Synthesis of coumarin 3-(*N*-aryl) sulfonamides was accomplished either by Knoevenagel condensation of anilinosulfonylacetic acids with suitable salicylaldehydes or by the reaction of methyl anilinosulfonylacetates with substituted salicylaldehydes in presence of a catalytic amount of a base. All the compounds tested for antiproliferative activity in different cancer cell lines have shown GI_{50} values less than 100 μM.

The diverse biological activities of natural and synthetic coumarin derivatives as anticoagulants and antithrombotics are well known.[1] Some of the coumarin derivatives are also reported as triplet sensitizers,[2] anti-HIV[3] agents, lipid-lowering[4] agents, and antioxidants.[5] They have also been found to inhibit lipid peroxidation and to possess vasorelaxant,[6] anti-inflammatory,[7] and anticancer activity.[8] Many coumarin derivatives are also known as free radical scavengers[9] and two naturally occurring coumarins have been found to exhibit cytotoxicity against a panel of mammalian cancer cell lines.[10] Some coumarin-3-carboxamides were reported as potent inhibitors of proteases such as α-chymotrypsin (CT)[11–13] and human leukocyte elastase (HLE).[14–16] Recently, a new class of sulfonamides[17,18] has been used in the treatment of diseases arising from abnormal cell growth and proliferation. Most cancers are characterized by uncontrolled cell proliferation, lack of cell differentiation, and loss of contact inhibition, which confers upon the tumor cell a capability to invade local tissues and metastasize. Recently, a series of arylsulfonanilides has been reported as potent inhibitors of various cancer cells at very low concentrations.[19] Based on the diverse biological activities of the coumarins and aryl sulfonamides, we have designed and synthesized a series of novel compounds that have both coumarin and sulfonamide entities in one molecule and have evaluated them for their antitumor activity.

Although several 3-substituted coumarins are known in literature,[20–23] very little information is available on coumarin-3-sulfonamides. In earlier papers,[20–23] different authors have used the same procedure for the synthesis of coumarin-3-sulfonamides where they have condensed coumarin-3-sulfonyl chloride with different aromatic amines. This method of synthesis has a limitation in having a variety of substituents at different positions on both aromatic rings and may not be useful in creating a library of new broadly unsubstituted coumarin-3-arylsulfonamides. In this letter we report a novel route for the synthesis of coumarin-3-sulfonamides using the Knoevenagel type of condensation reaction.

The reaction of methyl-2-chlorosulfonyl acetate with aromatic amines (**1**) gave methyl anilinosulfonylacetates (**2**), which on hydrolysis yielded anilinosulfonylacetic acids (**3**). Condensation of anilinosulfonylacetic acids with substituted salicylaldehydes (**4**) in glacial acetic acid in the presence of a catalytic amount of benzylamine gave coumarin-3-sulfonamides (**5**) in quantitative yields[24a] (Scheme 1). Alternatively, coumarin-3-sulfonamides (**5**) were also prepared by reacting

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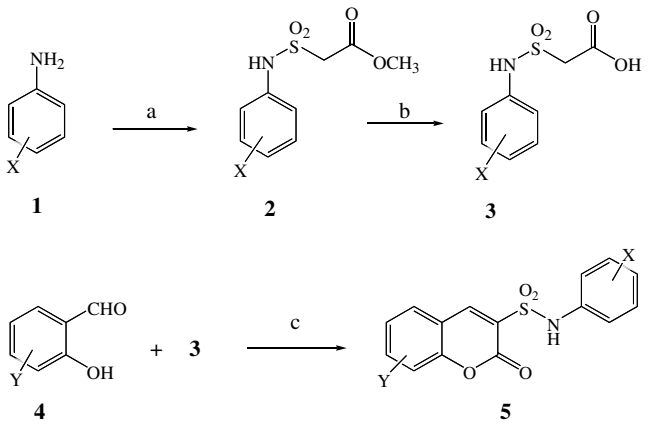
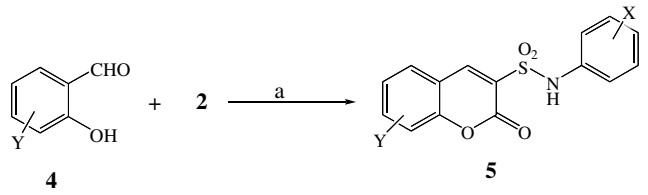


Scheme 1. Reagents and conditions: (a) $ClSO_2CH_2COOCH_3$, triethylamine/THF, N_2, rt, 3 h; (b) 10% NaOH in H_2O; (c) CH_3COOH, $C_6H_5CH_2NH_2$.



Scheme 2. Reagents and conditions: (a) piperidine, ethanol, reflux, 5 min.

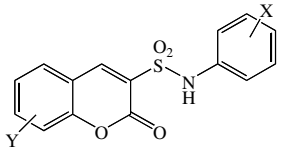
methyl anilino-sulfonylacetates with substituted salicylaldehydes in the presence of piperidine and ethanol[24b] (Scheme 2).

All the new compounds synthesized were characterized by ^1H NMR spectroscopy. Elemental analysis values for C, H, and N were within the range of the theoretical value. The ^1H NMR spectra of all new products **(5a–j)** showed a characteristic peak as a singlet at δ 8.50–9.20 for a methine proton ascertaining a cyclization reaction leading to the formation of coumarin-3-sulfonamides. A noncyclized Knoevenagel reaction would give an α,β-unsaturated compound with vinylic protons splitting as doublets at δ 7.3 and 7.6. Therefore, a singlet for a methine proton at δ 8.50–9.20 confirmed the cyclization reaction with the formation of coumarin-3-sulfonamides.

The effect of coumarin-3-sulfonamides on the growth of human tumor cells in culture was evaluated using androgen receptor negative prostate (DU145), colorectal (DLD-1), nonsmall cell lung carcinoma (H157), Estrogen receptor negative breast (BT20), and chronic myeloid leukemia (K562) cell lines. The dose response of each cell line was established by determining the number of viable cells after 96 h of continuous treatment against five different concentrations (1–100 μM range) of each compound. Table 1 shows the GI_{50} values, the concentration of each compound required to inhibit the growth of each tumor cell line by 50%, determined for each compound in the series.

Extra cellular signals received at transmembrane receptors are relayed into cells by the signal transduction pathways that have been implicated in induction of cell proliferation,[26] differentiation,[27] and apoptosis.[28] The mitogen activated protein kinase (MAPK) cascade is a major signaling system and many steps in this pathway are well conserved in different species. The best-studied MAPK family members are extra cellular signal-regulated kinases (ERKs)[29] and c-Jun NH_2 terminal kinases (JNKs).[30] JNKs are the members of the class of stress activated protein kinases (SAPK) and are shown to be activated by treatment of cells with UV radiation, proinflammatory cytokines and environmental stress.[31] It has been shown that activation of ERK involves phosphorylation of threonine and tyrosine residues by the upstream dual kinase and this activation signals cell proliferation where as the activation of JNKs eventually leads to cell growth inhibition and apoptosis.[32]

Table 1. Growth inhibition of cultured tumor cells



Compd	Cell line (GI_{50} μM)[a]						
	X	Y	BT20	DU145	H157	DLD-1	K562
5a	4-OMe	8-Br	16	15	18	20	14
5b	4-OMe	6-Cl	27	22	28	32	23
5c	4-OMe	8-OEt	52	57	74	78	48
5d	4-OMe, 3-OH	6-Cl	65	42	76	82	51
5e	4-F, 3-NH_2	8-OEt	54	48	72	80	42
5f	4-F, 3-NH_2	6-OMe	68	56	78	92	44
5g	4-Br	6-OMe	18	25	32	38	27
5h	4-Br	8-OEt	64	52	74	85	58
5i	4-Br	8-Cl	17	14	16	18	11
5j	4-Br	8-Br	12	12	15	14	16

[a] The GI_{50} was determined[25] by direct extrapolation from each dose response curve.

Since many types of cancer cells, following treatment with coumarin-3-sulfonamides, have shown considerable growth inhibition followed by cell death (Table 1), we evaluated the role of these compounds in activating Jun kinase signaling pathway. Some of the compounds, which have higher potency (**5a**, **5b**, **5g**, **5h**, and **5i**) were added to BT-20 cells at 50 and 100 μM concentration and harvested after 12 h. The cell lysates were then analyzed for c-Jun kinase activity[33] by immunoprecipitating JNK1 and assessing the ability of these immunoprecipitates to phosphorylate GST-linked c-Jun. Results[33] from these studies demonstrate that GST-c-Jun is hyperphosphorylated in all the cells that were treated with coumarin-3-sulfonamides compared to the DMSO treated controls (Fig. 1).

Several authors have reported the role of activated JNKs in inhibition of cell growth and death.[28,34,35] This pathway involves extra cellular signal-regulated kinases, which constitutes the PAK/MEKK/SEK/JNK kinase cascade, which ultimately phosphorylates c-Jun that is involved in apoptotic pathway. The activation of JNK1 by coumarin-3-sulfonamides as shown in immune complex kinase assay (Fig. 1), clearly shows that these compounds activate JNK pathway either by interacting with JNK1 or with one of the upstream kinases in this pathway. Current efforts are focused on identifying the target kinase for these compounds.

In summary, we report here a series of new coumarin-3-sulfonamides prepared by a novel method and their ability to activate JNK1 and kill tumor cells in vitro. These results provide an approach to develop new potent coumarin sulfonamides that kill tumor cells by activating JNK pathway.

8-Bromocoumarin 3-(N-4-methoxyphenyl)sulfonamide (**5a**): Mp 182–184 °C; ^{1}H NMR (500 MHz, DMSO): 10.24 (br s, 1H), 8.71 (s, 1H), 8.18 (s, 1H), 7.87 (d, $J = 9.0$ Hz, 1H), 7.41 (d, $J = 9.0$ Hz, 1H), 7.04 (d, $J = 9.0$ Hz, 2H), and 6.79 (d, $J = 7.0$ Hz, 2H); C, H, N analysis: calcd C 46.84%; H 2.95%; N 3.41%. Found C 46.67%; H 2.74%; N 3.36%.

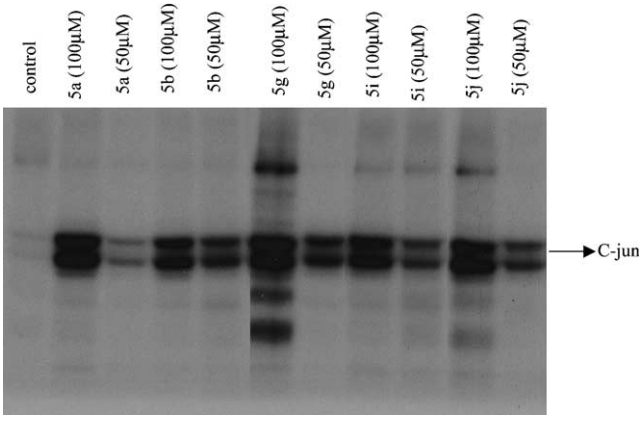


Figure 1. Activation of JNK-1 by coumarin-3-sulfonamides. BT-20 cell were treated with 50 and 100 μM of coumarin-3-sulfonamides and incubated for 12 h before cell lysis and immunoprecipitation with JNK1 antibody. The kinase activity of the immunoprecipitated JNK1 was tested using GST fused c-Jun (1-79 AA) as a substrate.

6-Chlorocoumarin 3-(N-4-methoxyphenyl)sulfonamide (**5b**): Mp 175–177 °C; ^{1}H NMR (500 MHz, DMSO): 10.24 (br s, 1H), 8.72 (s, 1H), 8.06 (s, 1H), 7.77 (d, $J = 9.0$ Hz, 1H), 7.49 (d, $J = 9.5$ Hz, 1H), 7.04 (d, $J = 7.0$ Hz, 2H), and 6.79 (d, $J = 7.0$ Hz, 2H); C, H, N analysis: calcd C 52.54%; H 3.30%; N 3.83%. Found C 52.33%; H 3.26%; N 3.77%.

8-Ethoxycoumarin 3-(N-4-methoxyphenyl)sulfonamide (**5c**): Mp 196–198 °C; ^{1} H NMR (500 MHz, DMSO): 10.18 (br s, 1H), 8.71 (s, 1H), 7.45 (d, $J = 8.0$ Hz, 1H), 7.40 (d, $J = 8.0$ Hz, 1H), 7.29 (d, $J = 8.0$ Hz, 1H), 7.05 (d, $J = 7.0$ Hz, 2H), 6.79 (d, $J = 7.0$ Hz, 2H), 4.13 (q, $J = 7.0$ Hz, 2H), 3.61 (s, 3H), and 1.36 (t, $J = 6.9$ Hz, 3H); C, H, N analysis: calcd C 57.59%; H 4.56%; N 3.73%. Found C 57.33%; H 4.38%; N 3.65%.

6-Chlorocoumarin 3-(N-4-methoxy 3-hydroxyphenyl)-sulfonamide (**5d**): Mp 225–226 °C; ^{1}H NMR (400 MHz, DMSO): 10.16 (br s, 1H), 9.14 (s, 1H), 8.76 (s, 1H), 8.12 (s, 1H), 7.81 (dd, $J = 8.8$, 3.2 Hz, 1H), 7.53 (d, $J = 8.8$ Hz, 1H), 6.77 (d, $J = 8.8$ Hz, 1H), 6.65 (d, $J = 2.5$ Hz, 1H), 6.55 (dd, $J = 8.8$, 2.5 Hz, 2H), and 3.67 (s, 3H); C, H, N analysis: calcd C 50.33%; H 3.17%; N 3.67%. Found C 50.18%; H 2.95%; N 3.61%.

8-Ethoxycoumarin 3-(N-4-fluoro 3-aminophenyl)sulfonamide (**5e**): Mp 160–162 °C; ^{1}H NMR (400 MHz, DMSO): 10.80 (br s, 1H), 8.71 (s, 1H), 7.54 (d, $J = 7.7$ Hz, 1H), 7.50 (d, $J = 7.7$ Hz, 1H), 7.42 (d, $J = 7.7$ Hz, 1H), 6.84 (d, $J = 8.8$ Hz, 1H), 6.60 (d, $J = 8.8$ Hz, 1H), 6.31 (m, 1H), 5.10 (br s, 2H), 4.28 (q, $J = 7.0$ Hz, 2H), and 1.51 (t, $J = 6.9$ Hz, 3H).

6-Methoxycoumarin 3-(N-4-fluoro 3-aminophenyl)sulfonamide (**5f**): Mp 255–260 °C; ^{1}H NMR (500 MHz, DMSO): 10.18 (br s, 1H), 8.53 (s, 1H), 7.52 (d, $J = 2.8$ Hz, 1H), 7.43 (d, $J = 9.0$ Hz, 1H), 7.33 (dd, $J = 9.0$, 2.8 Hz, 1H), 6.70 (d, $J = 8.5$ Hz, 1H), 6.47 (d, $J = 8.5$ Hz, 1H), 6.20 (m, 1H), 4.79 (br s, 2H), and 3.89 (s, 3H).

6-Methoxycoumarin 3-(N-4-bromophenyl)sulfonamide (**5g**): Mp 166–168 °C; ^{1}H NMR (400 MHz, DMSO): 10.88 (br s, 1H), 9.02 (s, 1H), 7.73 (d, $J = 2.5$ Hz, 1H), 7.60–7.50 (m, 4H), 7.27 (d, $J = 7.0$ Hz, 2H), and 3.95 (s, 3H); C, H, N analysis: calcd C 46.84%; H 2.95%; N 3.41%. Found C 46.70%; H 2.94%; N 3.12%.

8-Ethoxycoumarin 3-(N-4-bromophenyl)sulfonamide (**5h**): Mp 206–209 °C; ^{1}H NMR (400 MHz, DMSO): 10.75 (br s, 1H), 8.91 (s, 1H), 7.52 (d, $J = 7.6$ Hz, 1H), 7.45 (d, $J = 8.8$ Hz, 3H), 7.36 (t, $J = 8.0$ Hz, 1H), 7.13 (d, $J = 8.0$ Hz, 2H), 4.17 (q, $J = 7.0$ Hz, 2H), and 1.39 (t, $J = 6.9$ Hz, 3H); C, H, N analysis: calcd C 48.13%; H 3.32%; N 3.30%. Found C 47.60%; H 3.19%; N 3.21%.

8-Chlorocoumarin 3-(N-4-bromophenyl)sulfonamide (**5i**): Mp 202–204 °C; ^{1}H NMR (400 MHz, DMSO): 10.80 (br s, 1H), 8.89 (s, 1H), 8.13 (s, 1H), 7.81 (dd, $J = 8.8$,

2.8 Hz, 1H), 7.51 (d, $J = 8.8$ Hz, 1H), 7.44 (d, $J = 8.8$ Hz, 2H), 7.11 (d, $J = 8.8$ Hz, 2H), 6.79 (d, $J = 7.0$ Hz, 2H), 4.13 (q, $J = 7.0$ Hz, 2H), and 1.36 (t, $J = 6.9$ Hz, 3H); C, H, N analysis: calcd C 43.45%; H 2.19%; N 3.38%. Found C 43.39%; H 2.09%; N 3.21%.

8-Bromocoumarin 3-(N-4-bromophenyl)sulfonamide (**5j**): Mp 206–208 °C; ^{1}H NMR (400 MHz, DMSO): 10.80 (br s, 1H), 8.90 (s, 1H), 8.26 (s, 1H), 7.93 (d, $J = 9.0$ Hz, 1H), 7.45 (d, $J = 8.8$ Hz, 3H), 7.11 (d, $J = 8.8$ Hz, 2H), 7.05 (d, $J = 7.0$ Hz, 2H), 6.79 (d, $J = 7.0$ Hz, 2H), 4.13 (q, $J = 7.0$ Hz, 2H), and 1.36 (t, $J = 6.9$ Hz, 3H); C, H, N analysis: calcd C 39.24%; H 1.97%; N 3.05%. Found C 38.87%; H 1.89%; N 2.96%.

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24. (a) General experimental procedure for the synthesis of title compounds: Into a R.B. flask were added anilinosulfonyl acetic acid (1 equiv), salicylaldehyde (1 equiv) dissolved in acetic acid (10 mL), and a catalytic amount of benzyl amine (0.001 equiv), and the contents were refluxed for about 8–12 h in an oil bath. After cooling, the precipitated product was filtered and washed with isopropanol and diethyl ether. The pure products obtained were submitted for biological assay; (b) General method: To a solution of substituted salicylaldehydes (10 mmol) in warm absolute ethanol (20 mL) was added methyl anilinosulfonyl acetate (11 mmol) and three drops of piperidine. The solution was heated under reflux for 5 min. The crystalline product obtained after cooling was separated by filtration and washed three times with absolute ethanol to obtain a pure coumarin sulfonamide.
25. Cells (1×10^5) were plated into 6-well dishes and 24 h later each compound was added at five different concentrations over a 2 log dilution (1–100 μM). The total number of viable cells was determined after 96 h of continuous treatment by staining with trypan blue and counting the number of nonstaining cells (viable) remaining in each well using a hemacytometer. The percentage of viable cells remaining was calculated as follows: # viable cells (compound treated)/# viable cells (DMSO treated) $*$ 100. Dose response curves were generated by plotting the percentage of cells at each concentration versus concentration tested.
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33. The incubated cells with coumarin-3-sulfonamides at 37 °C were lyzed by lysis buffer [25 mM HEPES (pH 7.6), 0.1% Triton, 300 mM NaCl, 20 mM β-glycerophosphate, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 0.2 mM Na_3VO_4, 100 μM PMSF, 1 mM benzamidine, 2 μg/mL leupeptin, and 4 μg/mL aprotinin]. The protein concentrations were measured using Bio-Rad protein assay reagent. JNK1 in 100 μg of cell lysate was immunoprecipitated by incubating 100 μg of lysate protein with 1 μg of the JNK1 polyclonal antibody (Santa Cruz Biotechnology, Inc.,) for 1 h followed by an additional incubation with 20 μL of protein A-Sepharose (Pharmacia) for 1 h. The immune complex bound protein A-Sepharose beads were washed

twice with lysis buffer and twice with JNK buffer (20 mM HEPES, pH 7.6, 20 mM β-glycerophosphate, 10 mM $MgCl_2$, and 100 μM Na_3VO_4. The kinase reaction was carried out by resuspending the beads in 40 μL of JNK buffer containing 20 μM [γ-^{32}P]ATP (5000 CPM/pmol) and incubating them for 20 min at 30 °C using 5 μg GST-c-Jun as substrate. After stopping the reaction with the addition of Laemmli's buffer followed by boiling the samples for 3 min, the phosphorylated GST-c-Jun was separated on 12% SDS-PAGE. The gel was dried, and an autoradiogram was developed.

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