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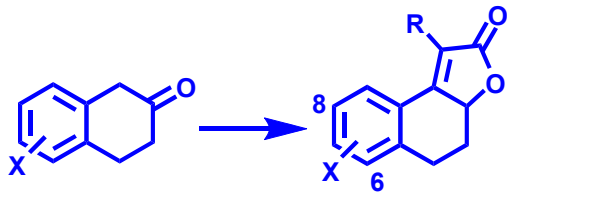
Table of Contents Entry

A one-step reaction gave naphtho[2,1-*b*]furan-2-ones which showed anti-inflammatory and breast cancer cell migration inhibitory effects.



2a: X = 6-OCH_3, R = CH_3
2b: X = 8-OCH_3, R = CH_3
2c: X = 8-OCH_3, R = C_6H_5

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Synthesis, microbial transformation, and pharmacological evaluation of 4,5-dihydronaphtho[2,1-*b*]furan-2-ones and related analogues

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Reaction of 5- or 7-methoxy-2-tetralone with an α-bromoester using lithium diisopropylamide as a base gave tricyclic naphtho[2,1-*b*]furan-2-ones in one step. Catalytic reduction, epimerization with triethylamine and microbial transformations yielded several related analogues. Some naphtho[2,1-*b*]furan-2-ones showed anti-inflammatory and breast cancer migration inhibitory activities.

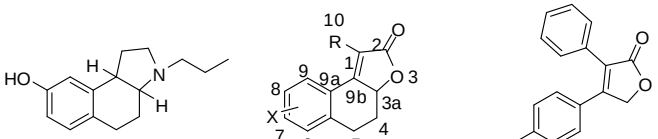
Introduction

As part of a program directed toward the discovery of ligands with selectivity at dopamine (DA) receptor subtypes, *cis*-(±)-2,3,3a,4,5,9b-hexahydro-8-hydroxy-3-*n*-propyl-1*H*-benz[*e*]indole (*cis*-(±)-8-OH-PBZI, **1**, Fig. 1) was shown to bind with high affinity and selectivity at DA D_3 receptors.[1,2] During the course of structure-activity relationship (SAR) studies with these benz[*e*]indoles, the synthesis of 1-substituted-hexahydro-1*H*-benz[*e*]indoles was initiated. We envisioned a reaction sequence similar to the one employed by Lin et al.[3] for the synthesis of hexahydro-1*H*-benz[*e*]indoles. Unexpectedly, reaction of a 2-tetralone with an α-bromoester using lithium diisopropylamide (LDA) as the base afforded tricyclic naphtho[2,1-*b*]furan-2-ones **2a-c** (Scheme 1).

During the course of this investigation, Pal et al.[4] reported the synthesis of conformationally restricted 3,4-diarylfuranones based on the naphthofuranone nucleus. These 4,5-dihydronaphtho[2,1-*b*]furan-2(3*aH*)-ones can be viewed as conformationally restricted 3,4-diarylfuranones that are structurally related to the COX-2 inhibitor rofecoxib (**3**, Scheme 1). Compounds **4a-4b** were prepared from appropriately substituted 1-tetralones by a three-step procedure. The naphtho[2,1-*b*]furan-2-ones **4a-b** (Scheme 2) exhibited comparable in vitro COX-2 inhibition as rofecoxib, although with less COX-2/COX-1 selectivity. Due to an increased risk of adverse cardiovascular events, rofecoxib was withdrawn from the market.[5]

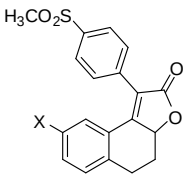
In an effort to study a greater diversity of compounds, the 1-substituted-4,5-dihydronaphtho[2,1-*b*]furan-2-ones **2a** and **2c** were reduced to the corresponding 1-substituted-1,4,5,9b-tetrahydrofuran-2-ones **10a** and **10b**, respectively. Lactone **2c** was transformed to corresponding lactam **16** by reaction with allylamine, and unsaturated lactones **2b** and **2c** were subjected to microbial biotransformation screening using a standard battery of microorganisms. The utilization of microbes as model systems to predict metabolic pathways in humans, to increase the efficacy of drugs by metabolic activation or to create novel and active analogs has been well documented.[6,7] This concept depends on the fact that fungi, being eukaryotes, possess metabolizing enzymatic machinery similar to those of mammals. Biotransformation and biocatalysis offer many distinct advantages, including: mild reaction conditions, highly stereo-, regio-, and chemoselective, unique and varied chemistry, and environmental safety.[6,7] Other advantages of biocatalysis for generating organic libraries include the natural diversity of enzymatic reactions, the compatibility of reaction conditions and high-throughput screening techniques, ease of automation, and the ability to retrace synthetic pathways leading to active products.[6,7]

The structural similarity of the naphtho[2,1-*b*]furan-2(3*a*)-ones and related derivatives to that of the COX-2 selective inhibitor rofecoxib, stimulated our interest in evaluating these compounds for anti-inflammatory activity. COX-2 is a key inducible enzyme controlling prostaglandin production, and inhibitors of COX-2 have been shown to induce apoptosis in various cancer cells.[8,9] Thus, several of the microbial metabolites and synthetic derivatives of the rofecoxib analogue **2c** were tested in a number of assays to assess anti-inflammatory and breast cancer antimigratory activity. The synthesis, characterization, and pharmacological evaluation of the 1-substituted-1,4,5,9b-tetrahydronaphtho[2,1-*b*]furan-2-ones and related derivatives will be described in this report.



1 (*cis*-(+/-)-8-OH-PBZI) **2a:** X = 6-OCH_3, R = CH_3 **2b**: X = 8-OCH_3, R = CH_3 **2c**: X = 8-OCH_3, R = C_6H_5 **3**: Rofecoxib

Scheme 1. Structures of PBZI (**1**), naphtho[2,1-*b*]furan-2-ones (**2a-c**), and rofecoxib (**3**).



4a: X = H
4b: X = CH_3

Scheme 2. Naphtho[2,1-*b*]furan-2-ones prepared by Pal et al.[4]

Results and discussion

1. Chemical synthesis

Reaction of 5-methoxy-2-tetralone (**5a**) or 7-methoxy-2-tetralone (**5b**) with an α-bromoester using LDA as the base afforded tricyclic naphtho[2,1-*b*]furan-2-ones **2a-c** (Scheme 3). Apparently, after initial alkylation of the 2-tetralones **5a** and **5b**, enolization of the ketoesters leads to the formation of the 4,5-

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dihydronaphtho[2,1-*b*]furan-2(1*H*)-ones **6a-c**, which after double migration during acidic work-up, affords the 4,5-dihydronaphtho[2,1-*b*]furan-2(1*H*)-ones **2a-c** (Scheme 3). Support for this mechanism is found in a study by Chavan and Govande[10] in which the formation of butenolide **8** from the diol **7** was reported using an excess of Amberlyst 15 (Scheme 4). When only a catalytic amount (10 %) of Amberlyst 15 was used, the ketoester **9** was the major product. These workers proposed that the ketoester was a possible intermediate in butenolide formation. This was confirmed by treating the ketoester with Amberlyst 15 in refluxing toluene to yield the butenolide **8**.

The structures of **2b**-**2c** were established based on extensive analysis of their 1D and 2D NMR data (Table 1). The methyl doublet H_3-10 in **2b** (δ_H 2.16) showed ^3J-HMBC couplings to the lactone carbonyl carbon C-2 (δ_C 175.2) and the downfield olefinic quaternary β-carbon C-9b (δ_C 156.2). It also showed a ^2J-HMBC coupling to the olefinic quaternary α-carbon C-1 (δ_C 119.4), confirming the α,β-unsaturated lactone formation. The oxygenated downfield proton H-3a (δ_H 4.49) showed ^3J-HMBC couplings to C-1, C-2, and C-5 (δ_C 27.0) and ^{1}H-^{1}H-COSY couplings with the methylene protons H_2-4 (δ_H 2.61 and 1.72) and H_3-10.

Catalytic hydrogenation of the unsaturated lactones **2a** and **2c** readily afforded the corresponding 1,4,5,9b-tetrahydronaphtho-[2,1-*b*]furan-2(3*a*)-ones **10a** and **10b** (Scheme 5). Hydrogenation of the 1,9b-double bond proceeded with *cis*-addition of hydrogen. The hydrogens at the C-1, 3, and 9b-positions are on the same side of the ring and are designated *cis-syn*.[1] Previously, single crystal x-ray crystallography of the structurally-related unsaturated tricyclic lactams showed that catalytic hydrogenation also yielded *cis-syn* reduction products.[11] Epimerization of the *cis*-syn lactone **10b** to the *cis-anti* diasteroisomer **11** was accomplished by stirring with Et_3N in CH_2Cl_2 for 48 h (Scheme 5). The structures of **10b** and **11** were confirmed by ^{1}H and ^{13}C NMR, HRMS, and elemental analysis.

2. Microbial transformations

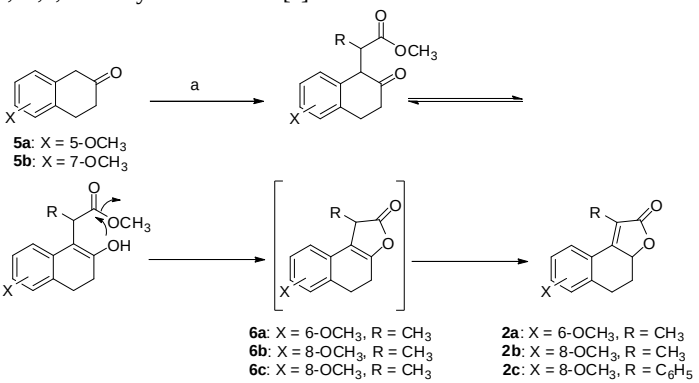
Twenty-eight growing microbial cultures were screened for their ability to bioconvert **2b** and **2c**. Of these, *Rhizopus arrhizus* ATCC 11145 was selected for the scale-up bioconversion of each compound. This selection was based on the TLC diversity of the generated metabolites. Biocatalysis of **2b** afforded two new metabolites, 5α-hydroxy and 1-hydroxymethyl analogs of **2b** (**12** and **13**, respectively) (Scheme 6). The naphthofuran analog **2c** was biotransformed by *R. arrhizus* to its 5α-hydroxy analogue (**14**) and benzeneacetamide (**15**).

The HREIMS data of **12** showed a molecular ion peak at *m/z* 242.0969 $[M+H]^+$, corresponding to molecular formula $C_{14}H_{15}O_4$. The ^{1}H and ^{13}C NMR data suggested a monohydroxy derivative of **2b**. The oxygenated methine carbon at δ_C 66.9 which correlated with the broad proton doublet at δ_H 5.09 was assigned C-5. This was based on the COSY coupling of H-5 with H2-4. The splitting pattern of H-5 and the small coupling value (J = 5.5 Hz) suggested its pseudoequatorial orientation. Therefore, the pseudoaxial C-5 hydroxy should be β-oriented. Hence, **12** was shown to be (5*R*)-5-hydroxy-8-methoxy-1-methyl-4,5-dihydro-naphtho[2,1-*b*]furan-2(3a*H*)-one. Similarly, metabolite **14** was found to be (5*R*)-5-hydroxy-8-methoxy-1-phenyl-4,5-dihydro-naphtho[2,1-*b*]furan-2(3a*H*)-one.

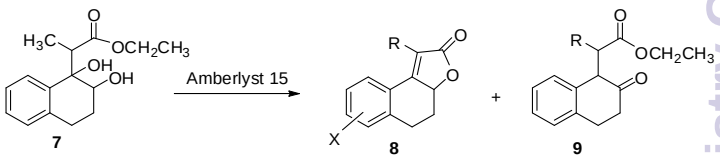
The HREIMS data of **13** showed a molecular ion peak at *m/z* 269.0789 $[M+Na]^+$, corresponding to molecular formula $C_{14}H_{14}O_4$. The ^{1}H and ^{13}C NMR data suggested a monohydroxy derivative of **2b**. The hydroxymethylene carbon at δ_C 55.6 which correlated with a geminally-coupled proton doublet pair at δ_H 4.76 and 4.54 (J = 13.2 Hz, each). This carbon was assigned C-10, which replaced the methyl H_3-10 in **2b**. Therefore, **13** was found to be 1-(hydroxymethyl)-8-methoxy-4,5-dihydronaphtho-[2,1-*b*]furan-2(3a*H*)-one.

Metabolite **15** was identified as benzeneacetamide based on its HRMS data (molecular ion peak at *m/z* 158.0579, $[M+Na]^+$ corresponding to the molecular formula C_8H_9NO, calculated 158.0576) as well as NMR data. This compound was identified as a biodegradation product of cyanohydrocarbons and diverse nitriles by the bacterium *Rhodococcus* strain RL4[12] and nitrile hydratase enzyme from the bacterium *Rhodopseudomonas palustris* CGA009.[13] This is the first report of this compound as a fungal biotransformation product.

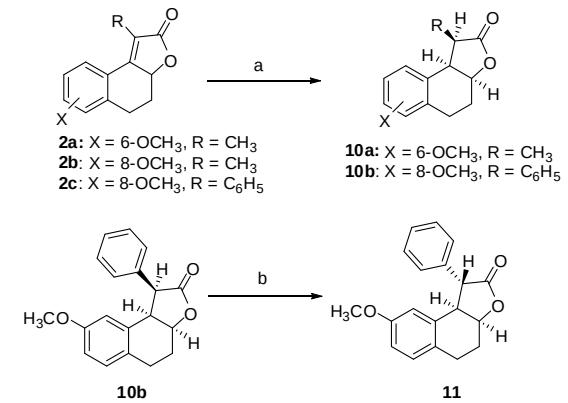
Reaction of **2c** in toluene with allylamine for 72 h at room temperature afforded the lactam **16** (Scheme 7). The HREIMS data of **16** showed a molecular ion peak at *m/z* 332.1639 $[M+H]^+$ corresponding to the molecular formula $C_{22}H_{22}O_2N$. The ^{1}H and ^{13}C NMR data (Table 1) suggested an *N*-allyl lactam derivative of **2c**. The upfield shift of C-3a (-17.5 ppm) in **16**, compared to its parent **2c**, indicated the replacement of the lactone oxygen with a lactam nitrogen. This was associated with additional new ^{1}H and ^{13}C NMR signals corresponding to an *N*-allyl group in **16** (Table 1). Therefore, **16** was shown to be 3-allyl-8-methoxy-1-phenyl-3,3*a*,4,5-tetrahydro-2H-benzo[*e*]indol-2-one.



Scheme 3. Synthesis of naphtho[2,1-*b*]furan-2-ones (**2a-c**). (a) LDA, $RCH(Br)CO_2CH_3$, THF.



Scheme 4. Intramolecular cyclization of diol **7** using Amberlyst 15.[10]



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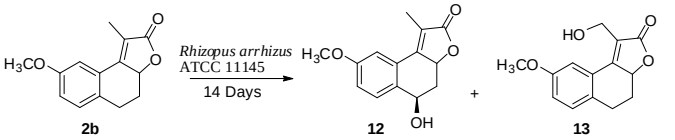
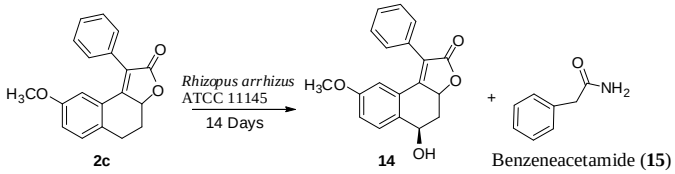
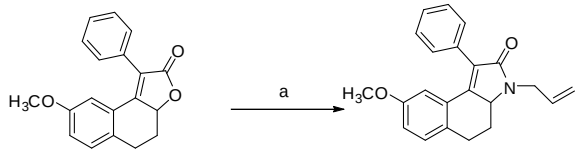
Scheme 5. Reduction of naphtho[2,1-*b*]furan-2-ones **2a-c** and epimerization of **10b**. (a) EtOH, 10% Pd/C, (b) CH_2Cl_2, Et_3N, 48 h.





Scheme 6. Biotransformation of the naphtho[2,1-*b*]furan-2-ones **2b** and **2c** using the fungus *Rhizopus arrhizus* ATCC 11145.



Scheme 7. Transformation of the naphtho[2,1-*b*]furan-2-one **2c** to the lactam **16**. (a) Allylamine, toluene, rt, 72 h.

3. Biological assays

3a. Anti-neuroinflammatory activity. The release of the eicosanoid thromboxane B_2 (TXB_2) and the free radical superoxide anion (O_2^-) by activated brain microglia (BMΦ) has been associated with several neuroinflammatory conditions.[14] *Escherichia coli* lipopolysaccharide-activated rat neonatal BMΦ have been extensively used as a convenient *in vitro* model to study the ability of compounds to inhibit production of neurotoxic TXB_2 and O_2.[15] Selecting a concentration range previously used with the *in vitro* microglia model[16], we investigated the effects of three concentrations (0.1 μM, 1 μM, and 10 μM) of compounds **2b**-**16** on the release of BMΦ O_2^- and TXB_2, and concomitant induction of lactate dehydrogenase (LDH) release, a well-established indicator of cellular cytotoxicity for *in vitro* pharmacological studies.[15] None of **2b**-**16** enhanced LDH release at the tested concentrations, except 10 μM concentration of compound **2a**, suggesting the potential toxicity of the 6-methoxynaphtofurans. Most compounds also did not inhibit BMΦ O_2^- and TXB_2 release even at 10 μM, the highest concentration tested. However, compound **2c** potently inhibited TXB_2 with an apparent IC_{50} of 1 μM, and did not affect O_2^- (Fig. SI1). This appears to be a pharmacological result because compound **2c** neither affected LDH nor superoxide anion generation. Furthermore, compound **16** potently inhibited both BMΦ O_2^- and TXB_2 with an apparent IC_{50} of 1 μM (Fig. SI2). Thus our current data support further development of **2c** and **16** as lead compounds for the development of novel anti-inflammatory agents to modulate activated brain microglia.[16]

3b. Growth inhibition effect of compounds 2b, **2c**, and **16 on the National Cancer Institute 60-human cancer cell lines.** The growth inhibitory activity of **2b**, **2c**, and **16** was evaluated by the National Cancer Institute's Developmental Therapeutics Program on a disease oriented panel. The panel consisted of 60-human cell line panel (NCI60), including nine different tissues (blood, lung, colon, CNS, skin, ovary, kidney, prostate, and breast). Unfortunately, the rest of compounds were not accepted for screening in this assay. Compounds were tested at a single concentration of 10 μM, and the percent growth of the 60 cell lines was determined and presented in the form of mean graphs (Figs. SI3-SI5, Table 3). These mean graphs provide insights into the differential cell growth inhibition for each compound, as well as a characteristic fingerprint pattern which possess a significant correlation to the compound's chemistry. Compounds **2b**, **2c**, and **16** showed mean percent growth of 73.9, 65.7, and 59.8, respectively, across all the 60 cell lines (mean-60, Figs. SI3-SI5). This proved significant growth inhibitory activities for these compounds with similar fingerprint of activity and were selective to cancer cells belonging to blood, prostate, skin, and breast tissues. C-1 phenyl group in **2c** showed better activity versus C-1 methyl group represented by **2b**. The *N*-allyl lactam analogue **16** was slightly more active than its parent **2c**, suggesting the preference of *N*-substituted lactam over the lactone group. Further SAR studies can prove the potential of 1-phenyl-3,3a,4,5-tetrahydro-2*H*-benzo[e]indol-2-ones as antiproliferative entity.

3c. Migration inhibition of compounds 2b-16 on the highly metastatic MDA-MB-231 human breast cancer cell line. The wound-healing assay is a simple method for the study of directional cell migration in vitro.[17] Cell migration is relevant to many cellular processes in morphogenesis, tissue repair, as well as cancer invasion and metastasis.[18] The ability of 4,5-dihydronaphtho[2,1-*b*]furan-2(3*aH*)-ones **2b**-**16** to inhibit the migration of the highly metastatic MDA-MB-231 human breast cancer cell line in wound-healing assay was evaluated (Fig. 1, Table 2). The doses used for the antimigratory assay were lower than the cytotoxic levels to avoid false positive activity due to possible cytotoxicity. Figure 1 shows cell migration across a wound inflicted in an MDA-MB-231 cell monolayer for the vehicle and positive controls. Compounds were tested at 2 different concentrations, 10 and 30 μM without any notable cytotoxicity. Most compounds showed antimigratory activity at both concentrations compared to vehicle control (Fig. 1, Table 2). The most active compound, the lactam **16** showed better activity at 10 μM, allowing only to 47.8% of MDA-MB231 cells to migrate, compared to 48.3% migration of the same cell with a 50 μM dose of the positive drug control (*Z*)-5-(4-[ethylthio]-benzylidene)-imidazolidine-2,4-dione (S-Ethyl, Table 2).[19-21] The 4,5-dihydro-naphtho[2,1-*b*]furan-2(3*aH*)-ones **2b**, **2c**, and their microbial metabolites **12** and **14** showed the next best antimigratory activity (Fig. 1). C-5 hydroxylation slightly improved the activity of **2b** and **2c**, based on the activity of **12** and **14** (Table 2). Meanwhile, C-1 hydroxylation slightly reduced the activity of **2b**. The 6-methoxy analgue **10a** and the simple benzeneacetamide metabolite **15** were among the least active, indicating the importance of 8-methoxy group and the 4,5-dihydro-naphtho[2,1-*b*]furan-2(3*aH*)-one skeleton, respectively, for the activity. The reduction C-1 epimeric product **11** had optimal activity, especially at 30 μM dose (Table 2), indicating no particular importance for $\Delta^{1,9b}$ system and the C-1 chirality for the antimigratory activity.

Conclusions

We have described the synthesis of 1-substituted-4,5-dihydronaphtho[2,1-*b*]furan-2-(3a)-ones **2a-c** in one step from methoxy-2-tetralones, an α-bromoester, and LDA. This is the first report of these compounds utilizing this method. In an effort to

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Table 1. ^{1}H and ^{13}C NMR Data of Compounds **2b**, **2c** and **16.**[a]

	2b		**2c**		**16**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	119.4, C		122.9, C		122.9, C	
2	175.2, C		173.3, C		170.8, C	
3a	79.0, CH	4.49, ddq (12.8, 4.8, 1.8)	77.5, CH	5.08, dd (12.8, 4.8)	60.0, CH	4.43, m
4	30.4, CH_2	2.61, dddd (13.5, 5.6, 4.8, 2.8) 1.72, dddd (13.5, 12.8, 6.0, 4.8)	30.3, CH_2	2.71, dddd (13.5, 5.6, 4.8, 2.8) 1.94, dddd (13.5, 12.8, 6.0, 4.8)	29.3, CH_2	2.56, m 1.66, m
5	27.0, CH_2	3.01, 2H, m	27.1, CH_2	3.06, 2H, m	27.5, CH_2	2.99, 2H, m
5a	129.6, C		128.2, C		129.2, C	
6	130.3, CH	7.17, d (8.4)	130.4, CH	7.14, d (8.4)	130.1, CH	7.15, m
7	116.5, CH	6.93, dd (8.4, 2.6)	118.9, CH	6.88, m	117.5, CH	6.94, m
8	158.2, C		157.7, C		157.4, C	
9	112.6, CH	7.15, d (2.6)	110.8, CH	6.87, m	111.0, CH	6.81, m
9a	130.1, C		130.6, C		132.5, C	
9b	156.2, C		157.5, C		149.3, C	
10	10.2, CH_3	2.16, 3H, d (1.8)				
8-OMe	55.5, CH_3	3.84, 3H, s	55.1, CH_3	3.47, 3H, s	54.9, CH_3	3.38, 3H, s
1`			128.9, C		129.8, C	
2`, 6`			128.7, CH	7.42, 2H, m	128.5, CH	7.42, 2H, m
3`, 5`			129.6, CH	7.45, 2H, m	129.9, CH	7.44, 2H, m
4`			128.8, CH	7.43, m	128.2, CH	7.43, m
N-Allyl					43.5, CH_2	4.12, m 3.94, m
					134.0, CH	5.90, m
					117.4, CH_2	5.22, 2H, m

[a] In $CDCl_3$, *J* in Hz. 400 MHz for ^{1}H and 100 MHz for ^{13}C NMR. Carbon multiplicities were determined by APT experiments, C = quaternary, CH = methine, CH_2 = methylene, CH_3 = methyl carbons.

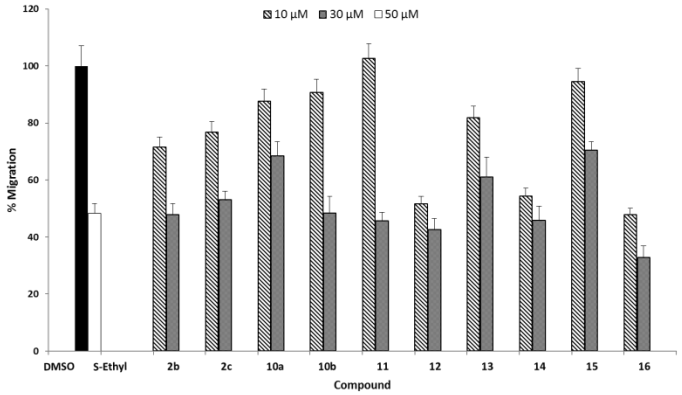

Fig. 1: Antimigratory activity of 10 and 30 μM doses of naphtho[2,1-*b*]furan-2-ones **2b**, **2c**, and **10-16** against the highly metastatic MDA-MB-231 human breast cancer cell line in wound-healing assay compared to DMSO vehicle control and 4-mercaptoethylphenylmethylene hydantoin (S-Ethyl) as a positive drug control.[19]

Table 2. Percentage Migration of Breast Cancer Cell Line MDA-MB231 after Treatment with 10 and 30 μM doses of Tested Compounds.

Compound	10 μM	30 μM
2b	71.4	47.8
2c	76.7	53.2
10a	87.6	68.4
10b	90.7	48.4
11	100	45.7
12	51.6	42.5
13	81.8	61.0
14	54.4	45.9
15	94.5	70.4
16	47.8	32.9
S-Ethyl[a]	48.3	-
DMSO	100.0	-

[a]4-Mercaptoethylphenylmethylene hydantoin (S-Ethyl) 50 μM dose was used as a positive drug control.[19]

increase the structural diversity, the lactones **2a-c** were chemically modified or subjected to microbial biotransformation. Three novel hydroxylated microbial metabolites (**12-14**) were obtained from lactones **2b** and **2c** with *Rhizopus arrhizus*. In the *in vitro* LPS-activated brain microglia assay, the phenyl-lactone **2c** potently inhibited TXB_2 with an apparent IC_{50} of 1 μM. Using this assay, the tricyclic lactam **16** inhibited both O_2^- and TXB_2 with apparent IC_{50} values of 1 μM. Substituted-4,5-dihydronaphtho[2,1-*b*]furan-2-(3a)-ones **2b**, **2c**, and the tricyclic

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lactam **16** showed excellent breast cancer migration inhibition and antiproliferative activity against several of the NCI-60 cell lines panel at 10-30 μM doses. The results show that the 1-substituted-4.5-dihydronaphtho[2,1-*b*]furan-2-(3aH)-ones are good candidates for further development as an anti-inflammatory and antimigratory agents.

Table 3. The Percent Growth of the Most Sensitive Cell Lines in Presence of 10 μM Dose of **2b**, **2c**, and **16**.

	2b	2c	16
Leukemia			
HL-60(TB)	74.17	52.99	34.92
K-562	54.16	42.06	28.27
RPMI-8226	20.66	18.14	11.53
Prostate Cancer			
PC-3	43.05	32.03	25.72
Melanoma			
MALME-3M	35.39	35.89	0.19
M14	63.67	64.90	46.68
SK-MEL-5	65.84	48.69	40.86
UACC-62	57.14	35.40	35.30
Breast Cancer			
MCF7	65.20	59.51	61.52
T-47D	64.30	30.92	40.67
MDA-MB-468	40.79	27.43	36.26

Experimental

General experimental procedures

IR spectra were recorded on a Varian 800 FT-IR spectrophotometer. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$, using TMS as an internal standard, on a JEOL Eclipse-400 NMR spectrometer, operating at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The HREIMS experiments were conducted at University of Mississippi on a Bioapex FTMS with electrospray ionization. Elemental analyses were obtained from Oneida Research Services, Inc., Whitesboro, NY. TLC analysis was carried out on precoated Si gel 60 F_{254} 500 μm TLC plates (EMD Chemicals), using *n*-hexane-ethyl acetate of $CHCl_3$-methanol as mobile phases. 1% Vanillin in concentrated H_2SO_4 was used as visualizing reagent. For column chromatography, Si gel 60 (E-Merck, 63-200 μm) was used.

Biotransformation. Biotransformation studies were conducted as described elsewhere.[22] Twenty-eight growing fungi cultures were screened for ability to biotransform **2b** and **2c**. These were: *Absidia spinosa* ATCC 6648, *Bauvaria bassiana* ATCC 7159, *Botyris allii* ATCC 9435, *Chaetomium globosum* ATCC 6205, *Cunninghamella elegans* ATCC 7929, *C. verticillata* ATCC 8986, *C. homothallica* ATCC 16161, *C. blakesleeana* ATCC 8688b, *C. blakesleeana* ATCC 9245, *Cunninghamella* sp. ATCC 201991, *Fusarium* sp. ATCC 11599, *Kluyveromyces africanus* ATCC 22294, *Lipomyces lipofer* ATCC 32371, *Lipomyces starkeyi* ATCC 58680, *Mucor ramannianus* ATCC 9228, *M. griseocyanus* ATCC 1207b, *Mucor* sp. ATCC 204009, *Nocardia acido*philus ATCC 22338, *N. corallina* ATCC 19148, *Phanerochaete chrysosporium* ATCC 24725, *Rhizopus arrhizus* ATCC 11145, *R. niveus* ATCC 200757, *R. oligosporus* ATCC 76011, *R. stolonifer* ATCC 6227a, *Saccharomyces pastorianus* ATCC 2366, *Streptomyces griseus* ATCC 13968, *S. malaysienesis* BAA-13 and *S. spheroides* ATCC 23965. *R. arrhizus* ATCC 11145 was selected for biotransformation scale-up and therefore was inoculated in twelve 1L flasks each containing 250 mL compound medium-α (20 g glucose and 5 g of each of peptone, yeast extract, NaCl, and Na_2PO_4 in 1 L distilled H_2O). After 48 h, a solution of 60 mg of **2b** and 400 mg of **2c** in EtOH was added to each flask (30 mg **2b**/flask and 40 mg **2c**/flask). After 14 days, the growth medium was filtered and extracted with EtOAc (1 × 1000 ml for **2b** and 4 x 1000 ml **2c**). The EtOAc layer was then concentrated under vacuum. Residue obtained from **2b** fermentation (130 mg) was purified on Si gel 60 column using isocratic $CHCl_3$-MeOH (9.5:0.5) to afford **12** (5.5 mg, R_f 0.60, $CHCl_3$-MeOH, 9.5:0.5, 17% yield), **13** (6 mg, R_f 0.39, $CHCl_3$-MeOH, 9.5:0.5, 19% yield) and recovered 28 mg non-metabolized starting material (**2b**). Similarly, purification of fermentation extract of **2c** (815 mg) on Si gel 60 using isocratic EtOAc-*n*-hexane (3:7) system afforded **14** (4.5 mg, R_f 0.65, EtOAc-*n*-hexane, 1:1, 2.6% yield), **15** (18 mg, R_f 0.17, EtOAc-*n*-hexane, 1:1, 10.6% yield), and recovered 230 mg non-metabolized starting material (**2c**).

6-Methoxy-1-methyl-4,5-dihydronaphtho[2,1-b]furan-2-(3a*H*)-one (2a). In a similar manner as described for **2b,** 5-Methoxy-2-tetralone (**5a**, 2.75 g, 15.6 mmol), lithium diisopropylamide (10.4 mL, 15.6 mmol, 1.5 M in cyclohexane), and methyl (±)-2-bromopropionate (2.87 g, 1.92 mL, 17.2 mmol) in THF (50 mL) produced a yellow semisolid. Trituration with hexanes afforded a yellow solid which was recrystallized from Et_2O-EtOAc to give 1.10 g (31%) of **2a**: mp 176-177 ºC; IR (neat) 2944, 2840, 1732, 1656, 1326, 1259, 1090, 791; ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 2H), 6.90 (d, 1H), 4.93 (m, 1H), 3.87 (s, 3H), 3.18 (m, 1H), 2.64 (m, 2H), 2.15 (d, *J* = 1.8 Hz, 3H), 1.66 (m, 1H); ^{13}C NMR δ 174.9, 157.5, 156.1, 129.9, 126.7, 119.7, 119.4, 111.7, 78.5, 55.6, 29.4, 21.8, 10.0; HRESIMS m/z 253.0820, $[M+Na]^+$ 253.0820 (calcd for $C_{14}H_{14}O_3Na$, 253.0835); Anal. Calcd for $C_{14}H_{14}O_3$: C, 72.02; H, 6.13. Found: 71.98; H, 6.00.

8-Methoxy-1-methyl-4,5-dihydronaphtho[2,1-*b*]furan-2(3a*H*)-one (**2b**). A solution of 7-methoxy-2-tetralone (**5b**, 1.00 g, 5.7 mmol) in THF (10 mL) was cooled to 0º and lithium diisoproylamide (3.78 mL, 5.7 mmol, 1.5 M in cyclohexane) was slowly added over 5 min. The mixture was stirred for 30 min and methyl (±)-2-bromopropionate (1.03 g, 0.69 mL, 6.2 mmol) was added. The solution was allowed to warm to room temperature and was stirred overnight. The reaction was quenched with 3 N HCl to pH < 3, and the THF was removed under vacuum. The residue was partitioned between CH_2Cl_2 (100 mL) and H_2O (3 x 100 mL), dried (Na_2SO_4), filtered, and evaporated to yield a yellow solid. Recrystallization from EtOAc-hexanes gave 0.47 g (32%) of **2b** as a white powder: mp 104-105.5 ºC; IR (KBr) 2945, 2837, 1747, 1653, 1493, 1321, 1170, 868 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); HRESIMS m/z 269.0585, $[M+K]^+$ (calcd for $C_{14}H_{14}O_3K$, 269.0575); Anal. Calcd for $C_{14}H_{14}O_3$: C, 73.02; H, 6.13. Found: C, 72.92; H, 6.20.

8-Methoxy-1-phenyl-4,5-dihydronaphtho[2,1-*b*]furan-2(3a*H*)-one (**2c**). Using the general procedure as described for **2b**, 7-methoxy-2-tetralone (**5b**, 1.00 g, 5.7 mmol), lithium diisopropylamide (3.8 mL, 5.7 mmol, 1.5 M in cyclohexane), and methyl (±)-2-bromophenylacetate (1.43 g, 0.98 mL, 6.2 mmol) in THF (10 mL) afforded a light brown solid. Recrystallization from EtOAc-hexanes gave 1.49 g (90%) of **2c** as a tan solid: mp 155-156.5 ºC; IR (KBr) 2945, 1745, 1647, 1562, 1485, 1155, 1036, 1004, 876 cm^{-1}; ^{1}H and ^{13}C NMR (see Table 1); HRESIMS m/z 293.1191, $[M+H]^+$ (calcd for $C_{19}H_{18}O_3$, 293.1172); Anal. Calcd for $C_{19}H_{18}O_3$: C, 78.06; H, 5.52. Found: C, 77.82; H, 5.46.

***cis-syn*-6-Methoxy-1-methyl-1,4,5,9b-tetrahydronaphtho[2,1-*b*]furan-2-(3a)-one (10a).** The unsaturated lactone (**2a**, 244 mg, 1.06 mmol) and 10% Pd/ˆC (100 mg) in 95% EtOH was hydrogenated on a Parr apparatus at an initial pressure of 40 psi. The catalyst was filtered, and the solvent was evaporated under reduced pressure to afford a white solid. Recrystallization from Et_2O-hexanes gave 121 mg (49%) of highly crystalline **10a**: mp

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72.5-73.5 ºC; IR (neat) 2949, 1756, 1583, 1334, 1258, 1172, 1134, 961, 752 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.15 (m, 1H), 6.69 (m, 2H), 4.97 (m, 1H), 3.83 (m, 3H), 3.82 (s, 3H), 2.77 (m, 2H), 2.17 (m, 1H), 1.86 (m, 1H), 1.03 (d, *J* = 8z, 3H); ^{13}C NMR ($CDCl_3$) δ 179.6, 157.3, 133.6, 127.0, 126.6, 123.0, 108.6, 77.7, 55.7, 41.5, 39.6, 27.5, 17.6, 13.8; HRESIMS m/z 255.0965 $[M+Na]^+$ (calcd for $C_{14}H_{16}O_3Na$, 255.0991; Anal. Calcd for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94. Found: 72.44; H, 6.95.

***cis-syn*-8-Methoxy-1-phenyl-1,4,5,9b-tetrahydronaphtho[2,1-b]furan-2-(3a)-one (10b).** In a similar manner as described for the synthesis of **10a**, the lactone **2c** (509 mg, 1.74 mmol) and 10% Pd/C in 95% EtOH (100 mL) gave 237 mg (46%) of **10b** as a white crystalline solid after recrystallization from Et_2O: mp 160-161 ºC; IR (neat) 2934, 1755, 1610, 1505, 1255, 1203, 1166, 953, 754 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.20 (m, 3H), 7.00 (m, 1H), 6.81 (m, 2H), 6.60 (m, 1H), 5.46 (d, *J* = 2.6 Hz 1H), 5.17 (brt, *J*= 3 Hz, 1H), 4.47 (d, *J* = 9.2 Hz, 1H), 4.00 (dd, *J* = 10,7 Hz, 1H), 3.18 (s, 3H), 2.92 (m, 1H), 2.51 (m, 2H), 1.76 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 176.90, 156.9, 134.14, 131.43, 130.88, 129.58, 129.28, 128.25, 127.62, 114.89, 114.60, 76.96, 54.85, 53.19, 44.63, 26.88, 22.39; HRESIMS m/z 333.0898 $[M+K]^+$ (calcd for $C_{19}H_{18}O_3K$, 333.0888; Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.39; H, 5.95.

***cis-anti*-8-Methoxy-1-phenyl-1,4,5,9b-tetrahydronaphtho[2,1-b]furan-2-(3a)-one (11).** The *cis*-syn-lactone **10b** (3.99 mg, 1.36 mmol) and Et_3N (550 mg, 5.44 mmol) in CH_2Cl_2 (15 mL) was stirred under nitrogen for 48 h. The solvent was evaporated to yield after recrystallization from 95% EtOH 248 mg (62%) of the *cis-anti*-lactone as a highly crystalline white solid: mp 130-132 ºC; IR (neat) 2947, 1759, 1611, 1499, 1266, 1142, 1003, 841, 748 cm^{-1}; ^1H NMR δ 7.39 (m, 1H), 7.05 (d, *J* = 8 Hz, 1H), 6.74 (dd, *J* = 8, 3 Hz, 1H), 6.17 (d, *J* =4 Hz, 1H), 5.01 (m, 1H), 3.76 (m, 2H), 3.73 (s, 3H), 2.74 (m, 1H), 2.18 (m, 1H), 2.07 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 176.63, 158.14, 137.0, 135.73, 129.80, 129.23, 128.93, 128.02, 127.55, 113.79, 113.21, 76.1, 54.79, 47.30, 27.28, 25.28; HRESIMS m/z 333.0889, $[M+K]^+$ (calcd for $C_{19}H_{18}O_3K$, 333.0888); Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16. Found: C, 77.29; H, 5.99.

(5*R*)-5-Hydroxy-8-methoxy-1-methyl-4,5-dihydronaphtho-[2,1-*b*]furan-2(3a*H*)-one (**12**). Brown semisolid, IR $(CHCl_3)\nu_{max}$ 2951, 1751, 1657, 1490, 1366, 1106, 965, 867 cm^{-1}; ^1H NMR: 5.39, ddq (12.8, 4.8, 1.8); 2.78, ddd (13.5, 4.8, 2.2), 1.83, ddd (13.5, 6.0, 4.8); 5.09, d (5.5); 7.02, d (8.3); 7.38, dd (8.3, 2.6); 7.15, d (2.6); 2.16, 3H, d (1.8); 3.87, 3H, s; ^{13}C NMR: 119.4, C; 175.8, C; 80.01, CH; 35.4, CH_2; 66.9, CH; 129.8, C; 131.3, CH; 117.5, CH; 158.3, C; 111.6, CH; 131.1, C; 156.2, C; 11.2, CH_3; 55.6, CH_3.; HRESIMS *m/z* 247.0969, $[M+H]^+$ (calcd for $C_{14}H_{15}O_4$, 247.0965).

1-(Hydroxymethyl)-8-methoxy-4,5-dihydronaphtho[2,1-*b*]furan-2(3a*H*)-one (**13**). Brown semisolid, IR $(CHCl_3)\nu_{max}$ 2950, 1750, 1657, 1490, 1366, 1106, 965, 867 cm^{-1}; ^1H NMR: 5.02, dd (12.8, 4.8); 2.66, m, 1.82, m; 3.04, 2H, m; 7.17, d (8.4); 6.98, dd (8.4, 2.6); 7.17, d (2.6); 4.76, d (13.2), 4.54, d (3.2); 3.83, 3H, s; ^{13}C NMR: 120.4, C; 175.5, C; 78.6, CH; 30.5, CH_2; 26.9, CH_2; 129.6, C; 130.3, CH; 118.3, CH; 160.2, C; 113.0, CH; 130.3, C; 158.2, C; 55.6, CH_2; 55.2, CH_3. HRESIMS *m/z* 269.0789, $[M+Na]^+$ (calcd for $C_{14}H_{14}O_4Na$, 269.0784).

(5*R*)-5-Hydroxy-8-methoxy-1-phenyl-4,5-dihydronaphtho[2,1-*b*]furan-2(3a*H*)-one (**14**). Brown semisolid, IR $(CHCl_3)\nu_{max}$ 2950, 1752, 1657, 1490, 1366, 1106, 965, 867 17 cm^{-1}; ^1H NMR: 5.52, dd (12.8, 4.8); 2.85, ddd (13.5, 4.8, 2.1), 1.72, ddd (13.5, 6.0, 4.8); 5.16, d (5.6); 7.34, d (8.4); 6.92, dd (8.4, 2.6); 6.99, d (2.6); 3.48, 3H, s; 7.44, 2H, m; 7.45, 2H, m; 7.46, m; ^{13}C NMR: 122.9, C; 173.2, C; 80.1, CH; 40.8, CH_2; 67.9, CH; 129.6, C; 131.4, CH; 116.4, CH; 158.2, C; 112.4, CH; 130.1, C; 156.2, C; 54.9, CH_3; 128.9, C; 128.7, CH; 129.6, CH; 128.8, CH; HRESIMS *m/z* 331.0945, $[M+Na]^+$ (calcd for $C_{19}H_{16}O_4Na$, 331.940).

Reaction of allylamine with 2c. Preparation of 3-allyl-8-methoxy-1-phenyl-3,3a,4,5-tetrahydro-2*H*-benzo[e]indol-2-one (16). A solution of 50 mg of **2c** in 2 mL toluene was added to 1 mL allylamine.[23] The reaction mixture was stirred for 72 h at rt. A brine solution (5 mL) was then added and the solution was extracted with $CHCl_3$ (2 x 10 mL). The organic layer was washed with H_2O (2 x 10 mL), dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The residue (167 mg) was fractionated by MPLC on Si gel 60 (10 g) using $CHCl_3$-MeOH, gradient elution to afford **16** (17 mg, R_f 0.45, $CHCl_3$-MeOH 9:1, 34% yield).

Anti-inflammatory Assays. Rat neonatal microglia (2 x 10^5 cells/24-well cell culture cluster) were stimulated with *Escherichia coli* lipopolysaccharide (LPS) (0.3 ng/mL) in 1 mL Dulbecco's modified Eagle medium, with 10% fetal bovine serum, penicillin, and streptomycin, for 17 h in a humidified 5% CO_2 incubator at 35.9 °C.[15-16] The medium was then removed and the microglia cells were washed with warm (35.9°C) Hanks' balanced salt solution (HBSS) and then incubated with compounds **2b-16** (0.1, 1 and10 μM) or vehicle (DMSO) for 15 min prior to stimulation with phorbol 12-myristate 13-acetate (PMA) (1 μM) for 70 min. All experimental treatments were run in triplicate and in final volume of 1 mL. O_2^-, TXB_2 and LDH release were determined as described elsewhere.[14,15]

NCI's 60-Cell line panel growth inhibition assay[24]

The NCI's human 60-cell lines were grown in RPMI 1640 medium containing 5% FBS and 2 mM L-glutamine. Cells were inoculated into 96-well plates at plating densities 5,000-40,000 cells/well, based on the doubling time of individual cell lines. Plates were then incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of tested compounds. After 24 h, two plates of each cell line were fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of tested compound addition. Tested compounds solubilized in DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. An aliquot of each frozen tested concentrate was thawed and diluted to twice the desired final maximum test concentration with complete medium containing 50 μg/mL gentamicin. 100 μL aliquot of tested drug dilution was added to appropriate wells containing 100 μL of medium, resulting in required final drug doses. Following tested compound addition, plates were incubated for additional 48 h. The assay was terminated by the addition of cold TCA for adherent cells. Cells were fixed *in situ* by addition of 50 μL of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min at 4 °C. Supernatant was discarded, and plates washed 5 times with water and air dried. Sulforhodamine B (SRB) solution (100 μL), 0.4% (w/v) in 1% acetic acid was added to each well, and plates incubated for 10 min at rt. After staining, unbound dye was removed by washing five times with 1% acetic acid and plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and absorbance was measured on plate reader at 515 nm. For suspension cells, the methodology was identical except the assay termination by fixing settled cells at the bottom of each well by adding 50 μL of 80% TCA (final concentration, 16% TCA).

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Antimigratory (wound-healing) assay[19-21]

The metastatic human breast cancer cells MDA-MB-231 cells purchased from ATCC were cultured in RPMI 1640 medium containing 10 mM HEPES, 4 mM L-glutamine, 10% FBS, penicillin (100 IU/mL), and streptomycin (50 µg/mL), and incubated in 5% CO_2 atmosphere at 37 °C and plated onto sterile 24-well plates and allowed to recover for a confluent cell monolayer formed in each well (>90% confluence) as previously reported.[19] Wounds were then inflicted to each cell monolayer using a sterile 200 µL pipette tip. Media were removed, cells were washed twice with PBS, and then test compounds added in fresh media (same culturing media but with only 0.5% FBS). Based on our optimization studies, 0.5% FBS was optimal to maintain appropriate survival and viability of MDA-MB-231 cells for reproducible wound healing assay, which was also consistent with literature.[17-19] A stock solution of each analogue was prepared by dissolving the compound in DMSO at 50 mM. About 2 µL of each stock solution was transferred to 998 µL of serum-free medium to obtain 100 µM concentrations (0.2% DMSO). Serial dilutions were then conducted to get the desired assay concentrations. Two non-cytotoxic concentrations (10 and 30 µM) of each compound were prepared in serum-free media containing 0.5% FBS. Each concentration was tested in triplicate. The negative control was prepared by adding 3 µL DMSO to 1497 µL serum-free media containing 0.5% FBS. (*Z*)-5-(4-[ethylthio]benzylidene)-imidazolidine-2,4-dione (S-Ethyl) was used as a positive drug control and DMSO as a vehicle control.[19,20] S-Ethyl was chemically synthesized by using base-catalyzed condensation of hydantoin with 4-ethylthio-benzaldehyde in aqueous ethanol in presence of catalytic amounts of $NaHCO_3$ and 2-aminoethanol and overnight reflux.[20] Incubation was carried out for 24 h, after which media was removed and cells were fixed and stained using DiffQuick™ staining kit (Dade Behring Diagnostics, Aguada, Puerto Rico). The number of cells migrated across the wound were counted under the microscope in 3 or more randomly selected fields (magnification: 40X). Final results expressed as % migration ([number of cells in the wound for treatment group/number of cells in wound for DMSO] x 100%) per 40X field.

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Notes and references

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† Electronic Supplementary Information (ESI) available: [Mean graphs showing the activity profile of the compounds on the National Cancer Institute 60 cell line panel]. See DOI: 10.1039/b000000x/

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