• FULL-LENGTH ARTICLES •

Novel Inhibitors of Rac1 in Metastatic Breast Cancer

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Objective: Rho family GTPases are molecular switches that control signaling pathways regulating a myriad of cellular functions. Rac1, a Rho family member, plays a critical role in several aspects of tumorigenesis, cancer progression, invasion, and metastasis. Rac proteins are not mutated in most invasive human cancers but are found to be overactive or over-expressed. Since Rho GTPases are activated by guanine nucleotide exchange factors (GEFs), inhibition of the interaction of Rac with its GEFs is a targeted strategy for blocking Rac activation.

Methods: The IC_{50} of NSC23766, an inhibitor of the interaction of Rac1 with a subset of GEFs, is too high for therapeutic use and more efficacious inhibitors are desired. Therefore, we initiated the synthesis of new derivatives of NSC23766 with modifications of the substituents connected to the central pyrimidine ring, and tested their Rac1 inhibitory activity.

Results: Several of the NSC23766 derivatives were shown to inhibit Rac1 activity of cancer cells with higher efficiency (20-50% more) than NSC23766. The new compounds are not toxic to normal mammary epithelial cells and are more efficient (60-70%) than NSC23766 in inhibiting cell migration and reducing cell spreading and extension of lamellipodia, cell functions regulated by Rac that contribute to cancer invasion.

Conclusion: Based on the results, we conclude that the novel compounds show promise of further development as small molecule inhibitors of invasive breast cancer progression. [*P R Health Sci J 2010;4:348-356*]

Key words: Rac1, GTPase, Inhibitor, Breast cancer, Metastasis

Breast cancer is the leading cancer type estimated for 2009 for US women and ranks first for death from all cancers at ages 20-59 (1). Early detection and aggressive breast cancer therapy in the US has resulted in a recent decline in mortality rates from breast cancer. However, in Puerto Rico, breast cancer mortality rates are rising and breast cancer has become the most frequently diagnosed form of cancer in women. Moreover, the recently released 2006 cancer statistics from the Centers for Disease Control and Prevention report that breast cancer is the number one killer of Hispanic women from all cancer related deaths. Breast cancer is most deadly when metastasis to other tissues occurs and novel therapies to inhibit spreading of the tumor are highly desired (2). We investigated Rac1 as a key protein involved in signaling pathways for metastasis, and synthesized novel small molecule compounds that can interfere with this process.

The involvement of Rac1 in metastatic processes

In metastasis, malignant cells migrate away from the original tumor site to other parts of the body. An important stage in the movement of these cells is the extension of actin-based protrusions by means of membrane ruffling and the formation of lamellipodia. Lamellipodia are sheet-like extensions of cross-linked networks of polymerized actin, and enable forward migration of the cells during invasion (3) and thus, regulate metastasis (4). A critical step for initiation of the cytoskeletal remodeling involved with lamellipodia formation is the activation of the small (21kD) GTP binding protein Rac1 (5). Therefore, inhibition of Rac1 activity could impede cell migration and provide a potential approach to prevent and halt metastasis (6).

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Rho GTPases

Rac1 belongs to the Rho family of GTPases, of which thus far, 22 members have been identified (7). Rho GTPases are important intracellular signaling proteins that control diverse cellular functions related to cancer development, including actin cytoskeleton organization, invasion and metastasis, transcriptional regulation, cell cycle progression, apoptosis, vesicle trafficking, and cell-to-cell and cell-to-extracellular matrix adhesions. Rho GTPases can be present in either a GDP-bound inactive conformation as well as in a GTP-bound active conformation, and is regulated mainly via GEFs (Guanine nucleotide exchange factors), GDIs (Guanine nucleotide dissociation inhibitors) and GAPs (GTPase activating proteins) (Figure 1) (8). When a GEF binds to a Rho GTPase in its GDP-bound inactive state, a molecule of GDP is exchanged for GTP, upon which a conformational change occurs that brings the Rho GTPase into an active state. For some RhoGTPases GDIs have been identified that inhibit this exchange of GDP for GTP. In its GTP-bound active conformation, the Rho GTPase can bind to its down-stream effectors, which mainly via activation of kinases leads to a biological effect. On the other hand, when a GAP interacts with active Rho GTPase, GTPase activity is stimulated and the bound GTP is hydrolyzed to GDP, thus reestablishing the Rho GTPase in its inactive form. Each member of the Rho GTPase class is regulated by one or more GEFs or GAPs.
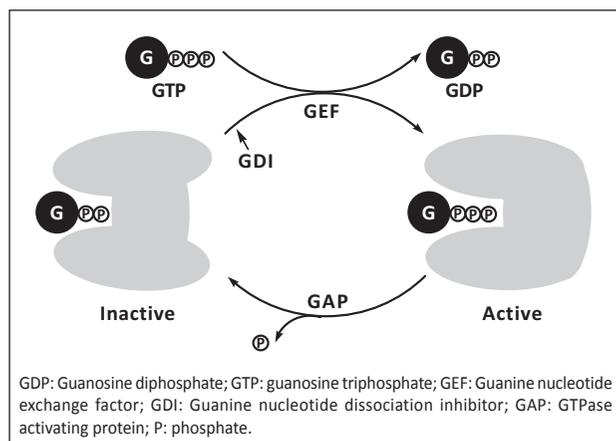

GDP: Guanosine diphosphate; GTP: guanosine triphosphate; GEF: Guanine nucleotide exchange factor; GDI: Guanine nucleotide dissociation inhibitor; GAP: GTPase activating protein; P: phosphate.

Figure 1. Regulation of Rho GTPases with different regulators

Rac1 as a target for drug discovery

The three main Rho GTPases are Rac1, Cdc42 and RhoA, and each has been shown to be specifically involved in lamellipodia formation, filopodia formation and stress fiber formation respectively (3,7). For both Rac1 and Cdc42, evidence has been obtained for their role in tumor growth, invasion and cell migration in breast tumors. Alongside Trio and Vav (9), an important GEF of Rac1 is "T-cell lymphoma invasion and metastasis 1" (Tiam1), which is considered to be an invasion-inducing gene (10). Increased expression of Rac1

has been reported in breast, colon and lung tumors (11), while over-expression of Tiam1 has been reported in highly invasive breast tumors and colon carcinomas (12). Increased Trio, Vav1, and Tiam 1 expression has been associated with high grade breast tumors (13). In addition, we and others have implicated hyperactive Rac1 with increased proliferation and invasion of breast cancer (14). Therefore, prevention of the binding of Tiam1 to Rac1 appears to be an attractive approach to inhibit the formation of lamellipodia and cell migration implicated with metastatic tumor cells.

Identification of a lead structure

The crystal structure of Rac1 with its GEF Tiam1 has revealed specific binding site residues required for binding of Rac1 with Tiam1 (15). Recently, via a virtual screening procedure utilizing molecular docking of the NCI library in this binding site, the group of Zhang identified NSC23766 (compound 1, Figure 2) as a selective inhibitor of Rac1 (16). NSC23766 has been shown to inhibit Rac activity and cell invasion from a number of systems (17). However, in breast cancer, NSC23766 only inhibited the invasion of low metastatic human breast cancer cell lines and not high metastatic cells. As we were interested in compounds that interfere with metastatic processes, we investigated the Rac1 inhibitory activity of NSC23766 in the highly metastatic cancer cell line MDA-MB-435. In this cell line, even at a concentration up to 200μM of compound 1, only 40% inhibition of Rac1 activity was observed. Therefore, we set out to utilize 1 as a lead structure for the synthesis of new derivatives with the potential for improved Rac1 inhibitory activity.
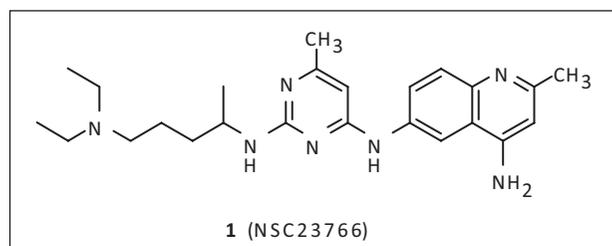

1 (NSC23766)

Figure 2. Lead structure for inhibitor design

Materials and Methods

Syntheses

Materials: All building blocks were purchased from Sigma-Aldrich Chemical company. **Procedures**: All novel NSC23766 derivatives were synthesized according to the reaction scheme provided in Scheme 1. As a representative example, the detailed two-step procedure for the synthesis of compound 7 is provided. **Step 1**: *Synthesis of (2-Chloro-6-methyl-pyrimidin-4-yl)-(2,3-dihydro-benzothiazol-6-yl)-amine (compound 8)*. To a solution of 1.63 g (10.0 mmol) 2,4-dichloro-6-methylpyrimidine in 10 mL iso-propanol, 1.97 mL (11.0 mmol) diisopropyl ethylamine

(DIPEA) and 1.65 g (11.0 mmol) 6-aminobenzothiazole were added. The solution was refluxed for 18 hours, after which the starting materials were shown to be consumed as detected by TLC (thin layer chromatography) or by GC/MS (Gas Chromatography with Mass Spectrometry detector). After the reaction mixture was cooled to room temperature, 20 mL ethyl acetate and 10 mL distilled water were added, and after vigorous mixing, the layers were separated with the aid of a separation funnel. The organic layer was extracted with 10 mL brine, separated and dried on sodium sulfate, filtered and concentrated on a rotary evaporator to obtain 2.49 g (9.0 mmol = 90%) of a crude yellowish solid. According to GC/MS a 3.0 : 1.0 mixture of respectively the 4-substituted and 2-substituted regioisomers was obtained. After silica gel chromatography using 3 : 1 hexanes/ethyl acetate as the eluent, the 4-substituted compound 8 was obtained as a pure regioisomeric compound in a yield of 1.11 g (4.0 mmol = 44% yield from crude material). The product was identified with NMR and GC/MS: ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 2.40 (s, 3H), 6.61 (s, 1H), 7.65 (d, J = 8.8 Hz, 1H) 8.11 (d, J = 10.5, 1H), 8.49 (s, 1H), 9.30 (s, 1H), 10.25 (s, 1H); ^{13}C (DMSO-d$_6$, 100 MHz) δ 23.2, 103.7, 112.9, 120.1, 12.9, 123.1, 149.2, 154.7, 158.9, 161.8, 167.3; LRGC-MS m/z (rel%): [M]$^+$ 276 (100), [M-Cl]$^+$ 241 (40), [M-C$_5$H$_7$N$_3$Cl]$^+$ 134 (26). **Step 2**: *Synthesis of N4-Benzothiazol-6-yl-N2-(4-diethylamino-1-methyl-butyl)-6-methyl-pyrimidine-2,4-diamine* (compound 7). In the second step, in a 10 mL glass microwave tube, 0.28 g (1.0 mmol) of compound 8 obtained in step 1 was dissolved in 1.0 mL *sec*-butanol, and 522 μL (3.0 mmol) DIPEA and 400 μL (2.0 mmol) 2-amino-5-diethylaminopentane were added. The tube was capped, placed in a CEM microwave synthesizer and heated to 120 ºC for 30 minutes. After the reaction mixture was cooled to room temperature, 10 mL ethyl acetate and 5 mL distilled water were added, and after vigorous mixing, the layers were separated with the aid of a separation funnel. The aqueous layer was extracted three times with 10 mL ethyl acetate, and after combining the organic phases they were extracted with 5 mL brine. The organic phase was separated and dried on sodium sulfate, filtered and concentrated on a rotary evaporator to obtain an off-white solid that was purified via silica gel chromatography to obtain 0.32 g (0.81 mmol = 81%) of product 7. The product was identified to be essentially pure by NMR and GC/MS analysis: ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 1.10 (t, J = 7.1 Hz, 9H), 1.35 (d, J = 6.4 Hz, 3H), 1.5-1.8 (m, 2H) 2.80 (m , 1H), 3.40 (s, 1H), 4.15 (m, 2H), 6.0 (s, 1H), 7.65 (d, J = 8.6 Hz, 1H), 7.96 (d, J = 8.8 Hz, 1H), 8.7 (s, 1H), 9.11 (s, 1H); ^{13}C (DMSO-d$_6$, 100 MHz) δ 9.9, 21.75, 22.8, 23.6, 35.5, 47.5, 48.2, 53.3, 96.5, 113.2, 121.1, 123.7, 135.9, 140.2, 149.5, 155.0, 163.2, 166.8; LRGC-MS m/z (rel%) [M]$^+$ 398 (4), [M-CH$_2$CH$_3$]$^+$ 369 (6), [M-C$_5$H$_{12}$N]$^+$ 86 (100).

Cell culture

MDA-MB-435 human metastatic breast cancer cells and MCF-10A mammary epithelial cells were purchased from ATCC and cultured in 10% DMEM at 5% CO2 and 37ºC incubator as described in (14c,d). The origin of MDA-MB-435 cell line has been questioned by comparative genomic hybridization studies that report MDA-MB-435 and M14 melanoma to be identical cell lines. However, as reviewed in (18), both cell lines may be of MDA-MB-435 breast cancer origin rather than of melanoma origin due to the following rationale. The MDA-MB-435 cell line was isolated from a pleural effusion of a female patient with breast cancer and still has two X chromosomes; expresses milk proteins and lipids; and when transfected with the nm23 metastasis suppressor gene, MDA-MB-435 cells show the morphologic features of normal breast epithelial cells, including acinus formation in three-dimensional culture. Therefore, the MDA-MB-435 cell line was used as a model for a highly invasive and metastatic breast cancer with high Rac activity.

Rac activity assay

MDA-MB-435 cells were treated with vehicle (0.1% DMSO), or 50 μM Rac Inhibitor for 24 hrs, and total protein extracts were obtained using the lysis protocol and buffers in the G-LISA Rac1 Activation Assay (Cytoskeleton, Inc., Denver, CO). As per manufacturer's instructions, Rac activity was measured in a 96 well plate coated with the Rac.GTP binding domain of p21-activated kinase (PAK) using the GLISA colorimetric assay.

Cell viability assay

Plates were seeded on a 96 well plate at a concentration of 2,000 cells/well in 100 μl of Phenol Red (-) DMEM with 5% fetal bovine serum (FBS). Cells were incubated in vehicle (0.1% DMSO) or Rac inhibitors at 50 μM for 24 hrs. Cell viability was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] cell survival and proliferation kit (Millipore, Inc., Billerica, MA). Absorbance at 600 nm of each experimental sample (3 biological repeats with 7 technical repeats), was measured from a 96 well plate on a Microplate Reader (Bio-Rad). The mean values ± SEM (N=3) are presented relative to vehicle (100%).

Cell migration assays

Quiescent MDA-MB-435 breast cancer cells were treated with vehicle (0.1% DMSO), or 50 μM Rac Inhibitor for 24 hrs. Cells were trypsinized and placed on the top well of Transwell chambers (Corning Life Sciences, Lowell, MA) in serum-free media with vehicle or 50 μM inhibitors. The bottom well contained 10% serum. The number of cells that migrated through 8μ pores in the membrane of the top well in response to various treatments was quantified relative to vehicle (100%) after 4 hrs. Data (±SEM) were quantified from analysis of 20 microscopic fields/treatment from 3 biological replicates.

Fluorescence microscopy

Quiescent GFP-tagged MDA-MB-435 cells were treated with vehicle (0.1% DMSO) or 50 μM Rac Inhibitors for 24 hrs. Cells

were fixed and stained for polymerized actin with Rhodamine Phalloidin as described in (14c). Representative microscopic fields are shown at 60X magnification.

Results and Discussion

Synthetic design

Compound 1 and analogous derivatives can be synthesized via successive connection of three chemical building blocks. The central building block is a pyrimidine core (A), which has been suggested to bind to a critical tryptophane (Trp56) residue of Rac1 (19). The second building block has a hetero-bicyclic arylamino group (B) connected to the 4-position of the pyrimidine ring, and the third building block is a primary or secondary aliphatic amine with a tail-end amino-group (C) connected to the 2-position of the pyrimidine ring. We developed a reaction scheme for the straightforward synthesis of a number of derivatives of compound 1 that allowed for variation in the building blocks (Scheme 1). In the first step, 2,4-dichloropymidine A is reacted with the arylamine B to give a 3:1 to 5:1 mixture of the 4-substituted and 2-substituted products. Via silica gel chromatography, the 4-substituted product is obtained purely, after which it is reacted with amine C to produce the desired novel derivatives of 1. The inhibitory activity of the new compounds towards Rac1 is determined and compared with the inhibitory activity of 1. The most active compounds were further investigated for their activity towards cell migration.
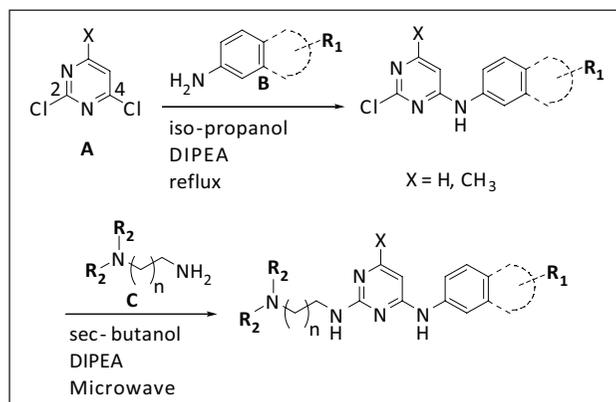
Scheme 1. Synthetic scheme for the preparation of analogues of NSC23766

Initial screening

All new compounds that were synthesized were tested for their Rac1 inhibitory activity in MDA-MB-435 metastatic breast cancer cells via a GLISA Rac1 activity assay. After incubation for 24 hours at a concentration of 50 μM, seven of the twenty compounds were shown to be more active inhibitors than NSC23766 (compound 1) in the screening for Rac1 inhibitory activity (Table 1). Seven other compounds

from the library also showed inhibition of Rac1, but to a lesser extent than NSC23766, while four compounds did not have any significant effect at all. Finally, two of the compounds appeared to be activators of Rac1. When analyzing the compounds grouped by the arylamino building blocks B, the following can be remarked: Of all compounds, the 6-aminoquinoline derivative 2 is structurally most related to compound 1, since it has both an identical amino-substituted aliphatic group C as well as a quinoline group in building block B as in 1. Nevertheless, this compound appears to be a much less active Rac1 inhibitor than 1. In contrast, compound 3, which is lacking the amino-substituted aliphatic group C and instead has a chloro-substituent, appears to be one of the most active inhibitors in this set of compounds. This result, combined with observations in the other compound groups, indicates that the presence of an amino-substituted aliphatic group C is not a strict requirement for inhibitory activity. For compound 4, a very modest activation of Rac1 is observed. The 5-aminoindole derivatives 5 and 6 both are Rac1 inhibitors that are somewhat more active than compound 1, whether an amino-substituted aliphatic side chain C is present (5) or not (6). Of the six 6-aminobenzothiazole derivatives, three (7, 11 and 12) were demonstrated to belong to the group of compounds that inhibit Rac1 to the largest extent of the currently available compound library. Interestingly, the other three compounds are less active (8) than NSC23766, or not significantly active at all (9 and 10). Of the four 5-aminobenzimidazole derivatives, three (13, 14 and 15) are much less active than 1, while compound 16 appears to activate Rac1 with 30% compared with vehicle. The 5-aminobenzopyrazole derivatives are either much less active Rac1 inhibitors (17, 18) or only slightly more active inhibitors (19) than NSC23766. The anilines 20 and 21 are both much less potent Rac1 inhibitors than compound 1. From the above results, it was decided to further investigate the active compounds from the 6-aminobenzothiazole class.

Effects of NSC23766 derivatives on cell viability

In order to establish whether the active aminobenzothiazoles 7, 11 and 12, as well as compound 1 demonstrated general toxicity, the cell viability of both the epithelial cell line MCF-10A and the highly metastatic breast cancer cell line MDA-MB-435 in the presence of these compounds were determined. In these assays, both cell lines were exposed to 50μM of the Rac1 inhibitors for 24 hours. As can be seen in Figure 3, the observed cell viability with compounds 7 and 11 is comparable to NSC23766 (1) and does not demonstrate substantial toxicity to either cell lines, although compound 11 seems to be somewhat more toxic to the MDA-MB-435 cell line than the other compounds. While relatively comparable, compound 12 appeared to have the greatest effect on cell viability in both cell lines, and was not further studied.

Table 1. Rac inhibition of novel derivatives compared with NSC23766 (1)

Quiescent MDA-MB-435 cells were treated with vehicle, or 50 μM Rac Inhibitor for 24 hrs, and total protein extracts were obtained. Rac activity was measured using G-LISA Rac1 Activation Assay (Cytoskeleton, Inc.). Average chemiluminescence of each experimental sample, was measured from a GTP-Rac affinity plate. Results are shown as the fold activity compared to Rac1 inhibition by the parent compound NSC-23766 (19.7% inhibition at 50μM).
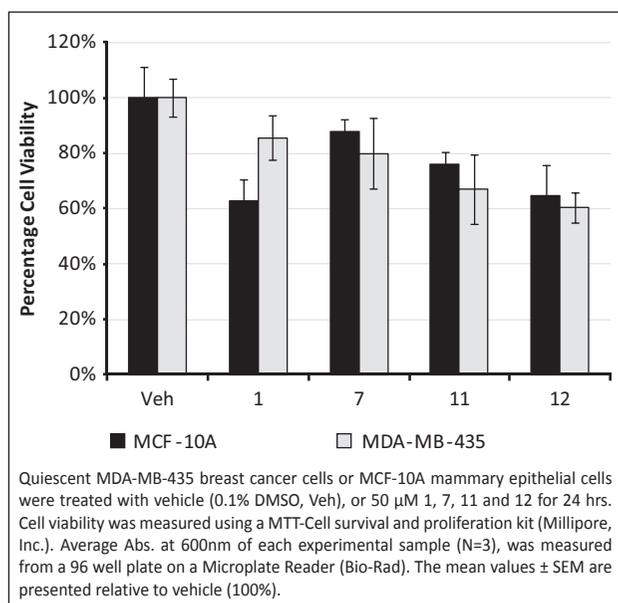
Quiescent MDA-MB-435 breast cancer cells or MCF-10A mammary epithelial cells were treated with vehicle (0.1% DMSO, Veh), or 50 µM 1, 7, 11 and 12 for 24 hrs. Cell viability was measured using a MTT-Cell survival and proliferation kit (Millipore, Inc.). Average Abs. at 600nm of each experimental sample (N=3), was measured from a 96 well plate on a Microplate Reader (Bio-Rad). The mean values ± SEM are presented relative to vehicle (100%).

Figure 3. Cell viability of MCF-10A and MDA-MB-435 in the presence of 1, 7, 11 and 12.

Quiescent MDA-MB-435 cells were treated with vehicle or 50 µM Rac inhibitors for 24 hrs and total proteins extracted from cell lysates. Rac activity was measured using G-LISA Rac1 Activation Assay. Results are shown as % inhibition where vehicle was set at 0%. The data are the mean of three technical replicates.
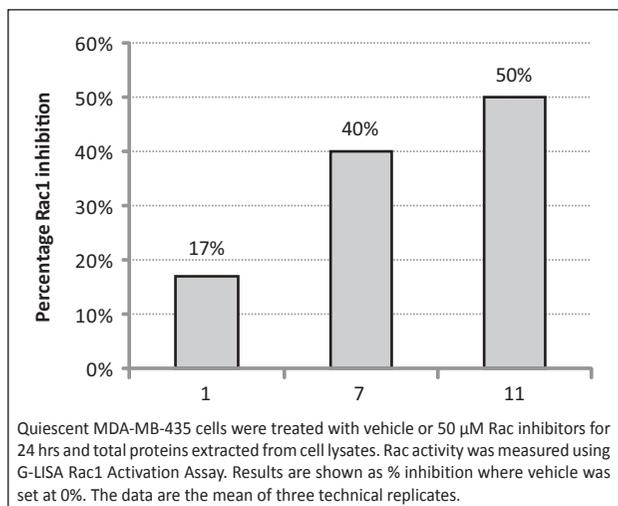
Figure 4. Inhibition of Rac1 activity by compounds 1, 7 and 11.

Effect of selected NSC23766 derivatives on Rac activity

Before further investigating the effects of the compounds of the 6-aminobenzothiazole class for their effects on cell migration and adhesion, we initially carried out a confirmatory assay of the Rac1 inhibitory activity of compounds 1, 7 and 11. A 24 h incubation of MDA-MB-435 cells with 50 µM NSC23766 resulted in only a 17% inhibition of Rac1 activity. As can be seen in Figure 4, compounds 7 and 11 gave 40% and 50% inhibition of Rac1 activity respectively under the same conditions. Therefore, this experiment confirms that NSC-23766 derivatives 7 and 11 are more efficient Rac1 inhibitors than the parent compound in the highly invasive cancer cell line MDA-MB-435.

Effects of selected compounds on cell shape and cell migration

We next tested the effect of compounds 1, 7 and 11 on cell shape, extension of cell surface actin structures called lamellipodia, and directed cell migration that are hallmarks of Rac-regulated cell functions. MDA-MB-435 cells were treated with vehicle or 50 µM 1, 7 and 11 for 24 h. From Figure 5 it can be observed that treatment of the cells with NSC23766 does not present a significant difference compared with vehicle. In contrast, compounds 7 and 11 are clearly much more efficient than NSC-23766 at reducing cell spreading and extension of lamellipodia, and directed migration towards serum. Compound 11 inhibited cell migration by about 60% more than parental compound 1 in a statistically significant manner. The effect of compounds 7 and 11 on cell migration was determined, and is represented in Figure 6. Interestingly, although at 50 µM concentrations these compounds inhibit Rac1 activity only by 40-50%, lamellipodia formation and cell migration is reduced by 80-90%. It is possible that *in vivo*, a 50% inhibition of Rac1 activity may be sufficient to exhibit the observed effects. Alternatively, 7 and 11 may have broader specificity and inhibit close Rac1 homologs such as Rac3 and Cdc42 that also regulate cell migration. Moreover, unlike the parental compound, these inhibitors may be active at different localized compartments of the cell as well as inhibit other upstream regulators of Rac. Rac
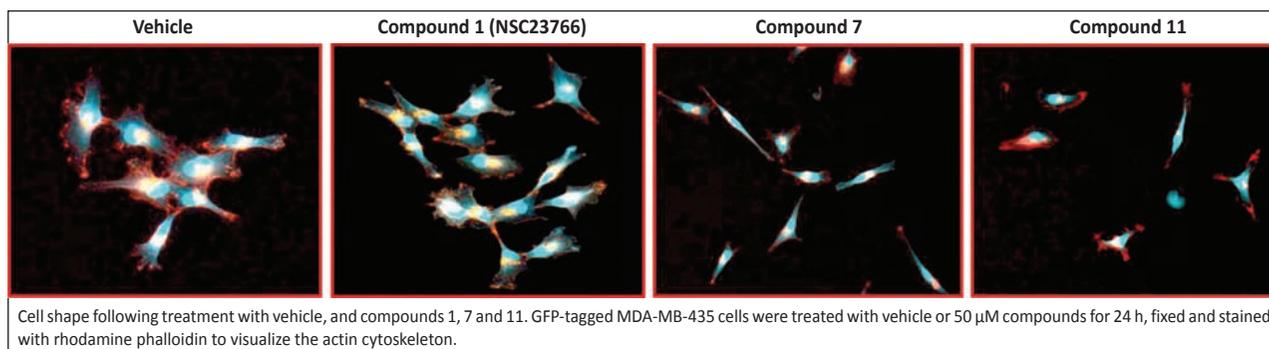

Cell shape following treatment with vehicle, and compounds 1, 7 and 11. GFP-tagged MDA-MB-435 cells were treated with vehicle or 50 µM compounds for 24 h, fixed and stained with rhodamine phalloidin to visualize the actin cytoskeleton.

Figure 5. Effects of compounds 1, 7 and 11 on lamellipodia formation

activity has recently been implicated in resistance to the anti Her2 therapeutic trastuzumab (20). Therefore, these novel anti Rac1 compounds have potential in combinatorial therapy to overcome resistance to common epidermal growth factor receptor-targeted therapies.
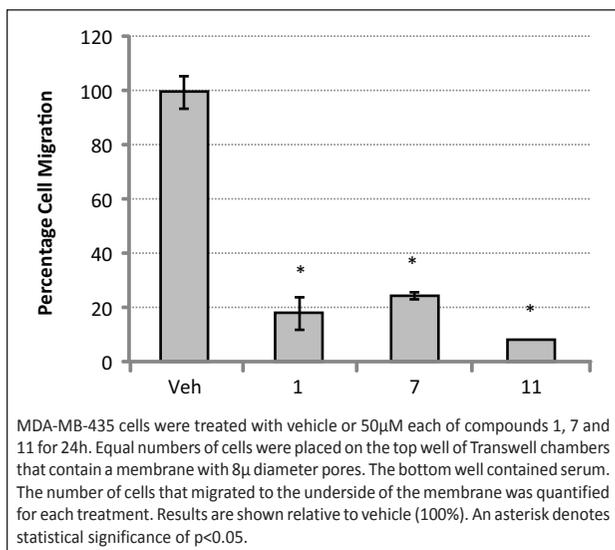

MDA-MB-435 cells were treated with vehicle or 50μM each of compounds 1, 7 and 11 for 24h. Equal numbers of cells were placed on the top well of Transwell chambers that contain a membrane with 8μ diameter pores. The bottom well contained serum. The number of cells that migrated to the underside of the membrane was quantified for each treatment. Results are shown relative to vehicle (100%). An asterisk denotes statistical significance of p<0.05.

Figure 6. Effects of compounds 1, 7 and 11 on cell migration

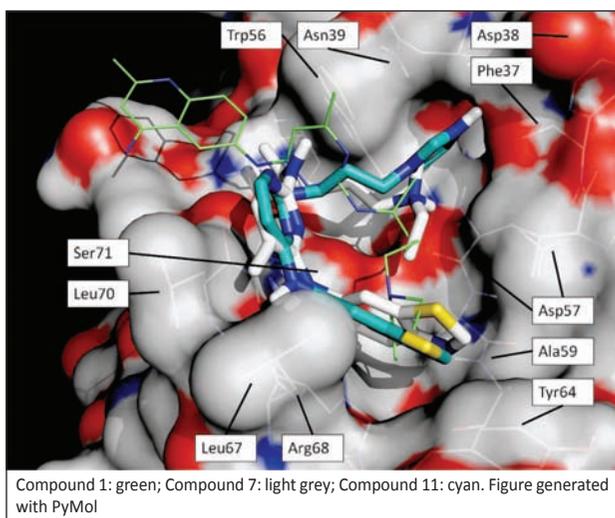Compound 1: green; Compound 7: light grey; Compound 11: cyan. Figure generated with PyMol

Figure 7. Compounds 7 and 11 docked into Rac1

Docking of 7 and 11 in the crystal structure of Rac1

The crystal structure of Rac1 with the inhibitor NSC23766 was recently revealed in a patent application (21). This structure has been used in a virtual screening of the ZINC database, and five new compounds with different core structures were identified as Rac1 inhibitors with IC50s from 12.2-57.2μM (22). Utilizing Autodock 4 (23), we now docked our novel Rac1 inhibitors 7 and 11 in this crystal structure. Similar as

the compounds identified from the ZINC database, both compounds 7 and 11 bind in multiple conformations of close energy within a binding pocket formed by amino acids Phe37, Asp38, Asn39, Trp56, Asp57, Thr58, Ala59, Tyr64, Leu67, Arg68, Leu70 and Ser71. The lowest energy conformation of the most populated cluster of the docking results of both compounds is represented in Figure 7, together with the position of compound 1 (green), in the crystal structure. Although NSC23766 (1) is stretched over the surface of Rac1, compounds 7 (light grey) and 11 (cyan), in all lowest energy conformations, including the ones shown, assume a bent shape and seem to dock deeper into the binding pocket. We will utilize docking in order to guide the design of inhibitors that will bind more tightly with Rac1.

Conclusion

Via minor structural modifications of NSC23766 we have identified two compounds (7 and 11) that are ~2-3 times more active as inhibitors of Rac1, while presenting minimal toxicity to epithelial cells. Both compounds were shown to have a much more dramatic effect on lamellipodia formation and cell migration than the lead compound NSC23766. Whether this is related to non-specific effects remains to be determined. Nevertheless, our experiments have demonstrated that the search for novel and more potent inhibitors of Rac1 can lead to compounds that are very effective in interfering with a key biological process related to metastasis. We expect that, utilizing molecular docking as an aid in the design, other compounds with increased efficacy can be synthesized and tested. Potentially, this could lead to a novel pharmaceutical treatment that prevents the spread of cancers to other tissues.

Resumen

Objetivo: La familia de Rho GTPasas son interruptores moleculares que controlan las vías de señalización y regulan una gran cantidad de funciones celulares. Rac1, un miembro de la familia de Rho, desempeña un papel crítico en varios aspectos de progresión del cáncer y metástasis. Rac no está mutada en cánceres humanos invasivos, pero se encuentra sobreexpresada. Debido a que las Rho GTPasas son activadas por factores de intercambio de nucleótido guanina (GEFs), inhibir la interacción de Rac con sus GEFs es una estrategia específica para el bloqueo de la activación de Rac. Métodos: El IC$_{50}$ de NSC23766, un inhibidor específico de Rac1 con un subconjunto de GEFs, es demasiado alto para uso terapéutico, por lo que inhibidores más eficaces son necesarios. Por lo tanto, iniciamos la síntesis de nuevos derivados del NSC23766 con modificaciones en los sustituyentes conectados al anillo central pirimidina, y examinamos su actividad inhibitoria contra Rac1. Resultados: Varios derivados de NSC23766

inhibieron la actividad de Rac1 en células cancerosas con mayor eficiencia (20-50% más) que NSC23766. Los nuevos compuestos no son tóxicos para las células epiteliales mamarias normales, pero son más eficientes (60-70%) que NSC23766 en la inhibición de migración celular y reducción de propagación y extensión de lamelipodia, funciones celulares controladas por Rac1 que contribuyen a la invasión de células cancerosas. Conclusión: Basado en los resultados, concluimos que los nuevos compuestos muestran un potencial de desarrollo como inhibidores de metástasis de cáncer de seno.

Acknowledgments

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