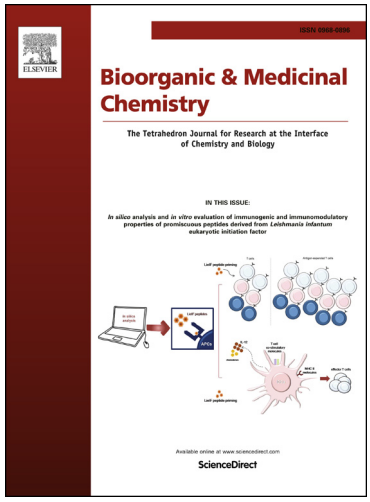
# Accepted Manuscript

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Cornelis P. Vlaar, Linette Castillo-Pichardo, Julia I. Medina, Cathyria M. Marrero-Serra, Ericka Vélez, Zulma Ramos, Eliud Hernández

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# Design, synthesis and biological evaluation of new carbazole derivatives as anti-cancer and anti-migratory agents

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**Abstract:** Based on the efficacy of EHop-016 as an inhibitor of migration and Rac1 activation, a new series of carbazole derivatives has been synthesized. Cytotoxic and anti-migratory effects of these compounds were evaluated in MCF-7 and MDA-MB-231 breast cancer cell lines. Preliminary investigations of their anticancer activity demonstrated that several compounds have moderate antiproliferative effects on cancer cell lines with $GI_{50}$ values in the range of 13-50 µM. Furthermore, compounds **3b** and **11b** inhibit migration activity of metastatic cell line MDA-MB-231 by 32% and 34%, respectively. Compound **11b** was shown to inhibit activation of the Rho GTPase Rac1 by 55% at 250 nM in both MDA-MB-231 and MDA-MB-435 cell lines. Compared with the $IC_{50}$ of Rac1 inhibition by lead compound EHop-016 of 1.1 µM, compound **11b** demonstrates 4X improved *in vitro* efficacy.

**Keywords:** Carbazole, EHop-016, Rac1, Breast Cancer, Migration, Metastasis, Rac1 inhibitor

## 1. Introduction

The major cause of death in breast cancer patients is the metastasis of primary tumor cells to secondary tissues. Early detection of breast cancer, prior to metastasis, provides patients with a higher probability of cure of their disease. To successfully invade a secondary site, a cancer cell completes a series of steps including migration from the primary tumor, invasion of surrounding tissues and basement membranes, entry (intravasation) and survival during circulation, and arrest at a distant target organ.[1] During cancer cell invasion, the migration of tumor cells through tissues frequently requires the degradation of the extracellular matrix (ECM). In this process, known as invadopodia formation, an array of several proteins play a key role.[2] Invadopodia are actin-rich protrusive structures associated with matrix degradation activity, and are believed to be important for tumor cells to be able to penetrate the basement membrane of epithelia and blood vessels.[2] The small GTPase Rac1, member of the Ras superfamily of GTPases, has been implicated in the regulation of cellular migration and invasion and invadopodia formation in breast cancer cells.[3-4] Rac1 is activated by GTP/GDP exchange factors (GEF) that are regulated via a myriad of cell surface receptors.[5] Therefore, therapeutic strategies that inhibit binding of GEFs to Rac1 are a rational means to inhibit migration of cancer cells.

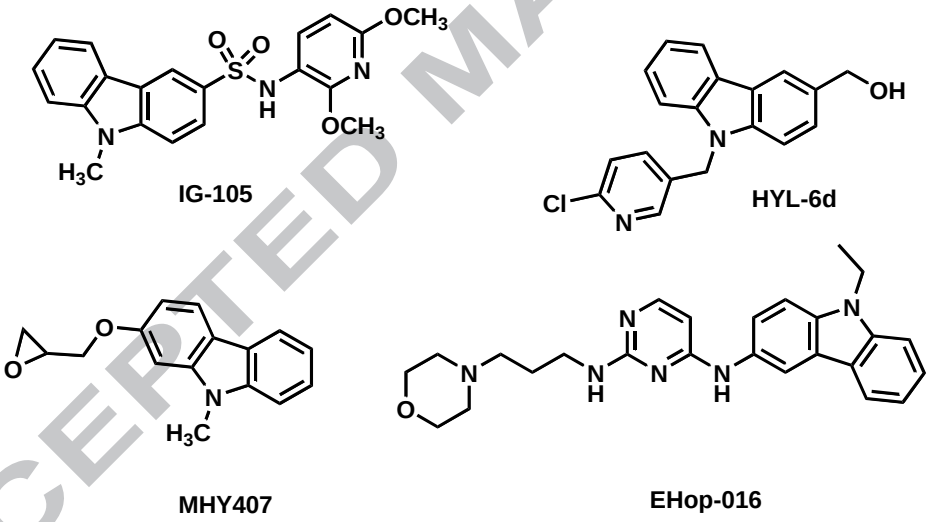



**Figure 1.** Structure of representative anti-cancer carbazole derivatives.

The carbazole skeleton is a key structural motif contained in a wide variety of synthetic and natural compounds with biological activities.[6,7] Carbazole derivatives have demonstrated diverse pharmacological activities including antioxidant,[7] anti-inflammatory,[8] antibacterial,[9] antitumor,[10-11] anticonvulsant,[12] antipsychotic,[13] antidiabetic,[14] and larvicidal[15] properties. The cytotoxic activity of carbazole alkaloids has been correlated to their polycyclic, planar aromatic structure.[6,16]

Selected examples of carbazole derivatives that have been evaluated for their anti-tumor potential against several human tumor cell lines are represented in figure 1. The carbazole sulfonamide IG-105 is an antimitotic agent that inhibits microtubule assembly through specific interactions within the tubulin structure.[17] Modelling studies suggested that the dimethoxypyridine and carbazole moieties bind to the hydrophobic pocket of tubulin, while the sulfonamide group and the *N* atom of the carbazole group form hydrogen bond interactions. Compound HYL-6d inhibits proliferation and migration in HUVEC cells under pathological angiogenic conditions, critical factors in breast cancer progression and metastasis.[18] The epoxypropoxy carbazole derivative MHY407 effectively causes DNA damage by C-PARP production, topoisomerase II inhibition and cell cycle arrest in the S phase by regulating cyclin D1, pRb, and p21 levels.[19] Our laboratory recently developed EHop-016, which was shown to reduce metastatic cancer cell viability at a concentration of $\leq$5 μM.[20] Specifically, EHop-016 inhibits Rac1-Vav2 interaction with an $IC_{50}$ = 1.1 μM. As a consequence, the Rac1-downstream effector PAK1 was inhibited by ~60% at 2 μM, leading to reduction of *in vitro* lamellipodia formation and cell migration. Its activity was as inhibitor of tumor growth and metastasis in was demonstrated in an *in vivo* mouse model of breast cancer.[21]

Molecular modeling suggested that EHop-016 binds to Rac1 via adoption of a "U-shaped" conformation.[20] We hypothesized that compounds with a more compact structural conformation that closely adopt this "U-shaped" conformation, could improve inhibitory activity against Rac1. Previous research has indicated that the carbazole group present in EHop-016 significantly contributes to Rac1 inhibitory activity. Therefore, we designed and synthesized several new series of EHop-016 derivatives that maintain the carbazole group while mimicking this "U-shaped" conformation. Their cytotoxic and anti-migratory activity against metastatic cancer cells was determined, and the most active migration inhibitor was further evaluated for its Rac1 inhibitory activity.

## 2. Results and discussion

The main goal of this project is to discover novel anti-metastatic agents as identified by their potential to inhibit cancer cell migration, while demonstrating limited off-target cytotoxicity. The carbazole and the morpholinopropylamine substituents of EHop-016 are in a 1,3-relationship with respect to the pyrimidine core (figure 1). It was reasoned, as also suggested via molecular dockings, that a 1,2-relationship would be more likely to adapt a U-conformation, hypothesized to be favorable for Rac1 binding. Therefore, several series of compounds were synthesized that replace the pyrimidine core with a pyridine, pyrazine, benzene or cyclohexane group. This strategy enables positioning of the carbazole group in an ortho-relationship with the second, modifying substituent. The synthetic procedures are described in schemes 1 to 3.

For all new compounds, the growth inhibitory activity against MCF-7 and MDA-MB-231 breast cancer cells were tested using the Sulforhodamine B (SRB) assay[22]. In addition, anti-migratory activity was determined using the scratch-wound healing assay[23]. In this assay, the relative migration of MDA-MB-231 breast cancer cells in the presence of the novel compounds at a concentration of 10 µM was compared to the migration in the presence of vehicle (0.02% DMSO). Representative photomicrographs of the migration inhibition of compound **3b** and **11b** (see later) are represented in figure 2. It can be observed that in the control, after 12 hours, wound healing is progressing considerably, and after 24 hours, the wound is basically healed. In contrast, in the presence of **3b** or **11b**, both after 12 and 24 hours, the wound healing is significantly inhibited. Migration can only be observed in the metastatic cell line MDA-MB-231, since the MCF-7 cells hardly migrate. The biological activities of the new compounds are summarized in tables 1 to 3.

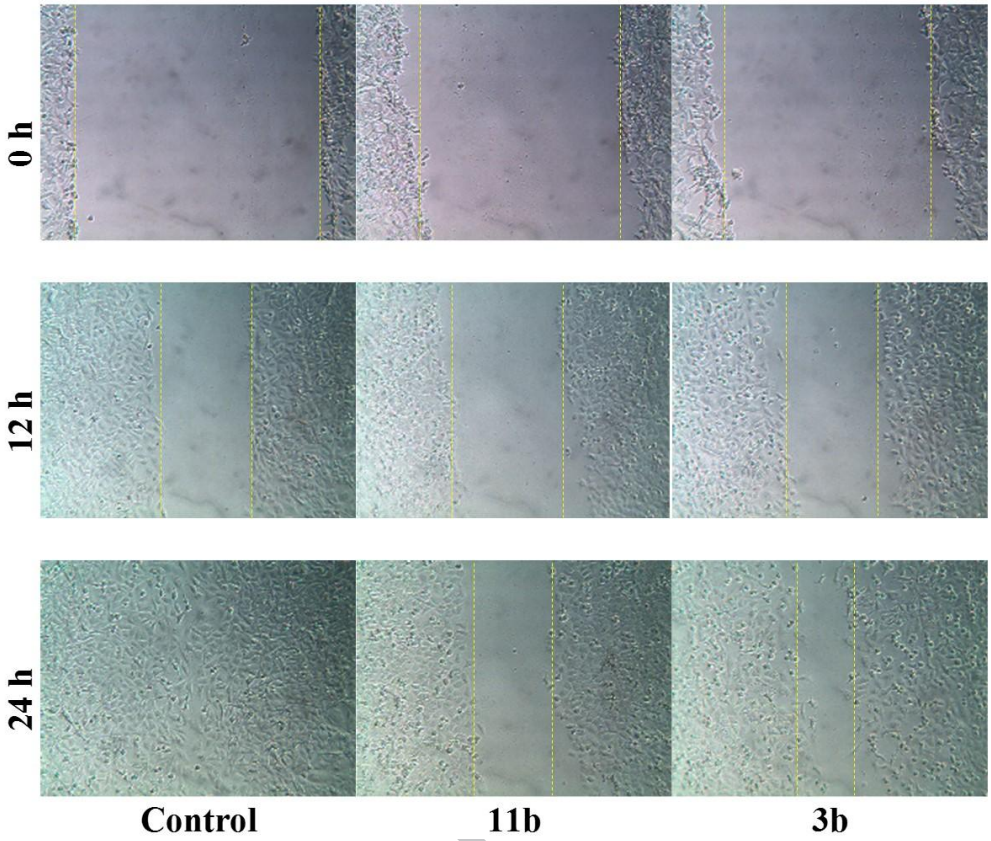
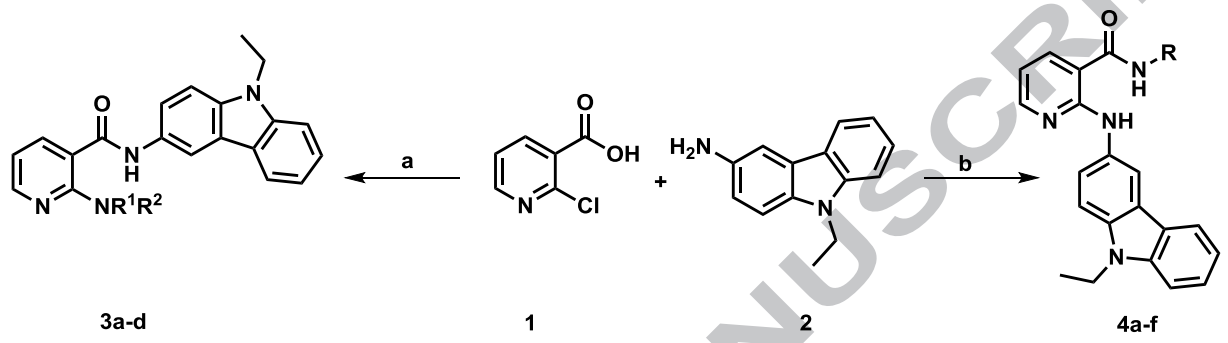



**Figure 2.** Inhibitory effect of **11b** and **3b** on MDA-MB-231 cells migration detected by wound-healing assay. MDA-MB-231 cells were treated with vehicle or with **11b** and **3b** at 10 μM and photomicrograph obtained at 0, 12, and 24 h.

### *2.1 Ortho amino-carboxamide-substituted EHop-016 derivatives*

In the first two series of novel compounds (**3a-d** and **4a-f**, scheme 1), we explored the introduction of ortho amino-carboxamide substituents via replacement of the core pyrimidine group of EHop-016 with a pyridine group. In these two series, the amino group is located at 2-position and the carboxamide group at the 3-position of the pyridine ring. The carbazole group either forms an aromatic amine at the 2-position or an amide at the 3-position. Both options place the key pharmacophores in a more compact ortho-substituted relationship, hypothesized to provide compounds with increased activity. Compounds **3a-d** were synthesized via amide coupling of 2-chloronicotinic acid **1** with carbazole **2**, followed by a CuI-catalyzed coupling reaction with different amines to afford the corresponding 2-aminonicotinamide derivatives **3a-d** (scheme 1a). Compounds **4a-f** were synthesized by nucleophilic aromatic substitution of

2-chloronicotinic acid **1** with carbazole **2** under microwave irradiation in water at 140 °C for 5 h in the presence of 3 equiv. DIPEA (scheme 1b). The intermediate 2-aminocarbazole-nicotinic acid was reacted via an amide coupling reaction with amines to obtain the corresponding 2-carbazolamine-nicotinamide derivatives.

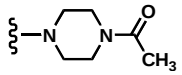
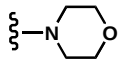
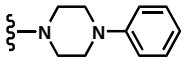
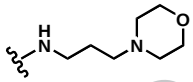
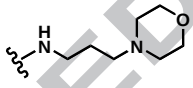
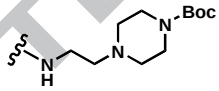
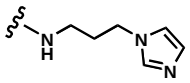
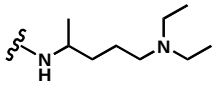
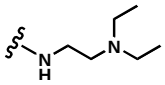
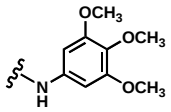



**Scheme 1.** General synthetic routes of 2-substituted-nicotinamide derivatives **3a-d** and **4a-f**. Reagents and conditions: (a) (i) HOBt, EDAC, DMF, $Et_3N$, rt; (ii) CuI, DIPEA, dioxane, 80 °C, 8-10 h, R = $HNR^1R^2$; (b) Method A (i) DIPEA (3 equiv), water, 140 °C, 5h, (ii) HOBt, EDAC, DMF, $Et_3N$, rt, R-$NH_2$. Method B (i) HOBt, EDAC, $CH_2Cl_2$, $Et_3N$, rt; (ii) CuI, $Cs_2CO_3$, DMSO, 90 °C, 24 h, R-$NH_2$.

From table 1 it can be observed that in the MCF-7 cancer cell line, compounds **4a** and **4c-e** showed moderate antiproliferative activity with a $GI_{50}$ in the range of 13.4-28.3 μM. In the MDA-MB-231 cell line, compounds **3d** and **4d-e** inhibited cell proliferation with a $GI_{50}$ in the range of 18-19.3 μM. The remaining compounds in both series had a $GI_{50}$ above 50 μM in both breast cancer cell lines tested. Thus, in general, compounds of the second series with the aminocarbazole group at the 2-position appear to be more cytotoxic than compounds in the first series. For comparison, the $GI_{50}$ of EHop-016 in the MCF-7 and MDA-MB-231 cell lines was 14 and 15 μM respectively. The relative migration compared with control of compounds **3a-d** and **4a-f** are summarized in table 1. Among the ten compounds available in these two series, none of the compounds of the second series **4a-f** inhibited migration significantly. While compounds **4d** and **4e** were among the most cytotoxic compounds tested, they did not significantly inhibit migration. In contrast, compound **3b** inhibited migration with 32%, comparable with parent compound EHop-016, which exhibited anti-migratory activity of 33% at 10 μM (and 17% at 5 μM). Compound **3d**,

in which a propylene group is inserted between the morpholine and pyridine ring of **3b**, shows a reduced activity against cell migration as well as moderate growth inhibitory activity against the MDA-MB-231 cell line. When comparing EHop-016 and compound **3b**, migration inhibition of MDA-MB-231 cells is comparable, but the $GI_{50}$ values are 15 µM and > 50 µM respectively. Therefore, compound **3b** exhibits reduced toxicity with similar anti-migratory potential compared to EHop-016.

**Table 1.** Growth inhibition and anti-migration activity for compounds **3a-d** and **4a-f** on MCF-7 and MDA-MB-231 cell lines.

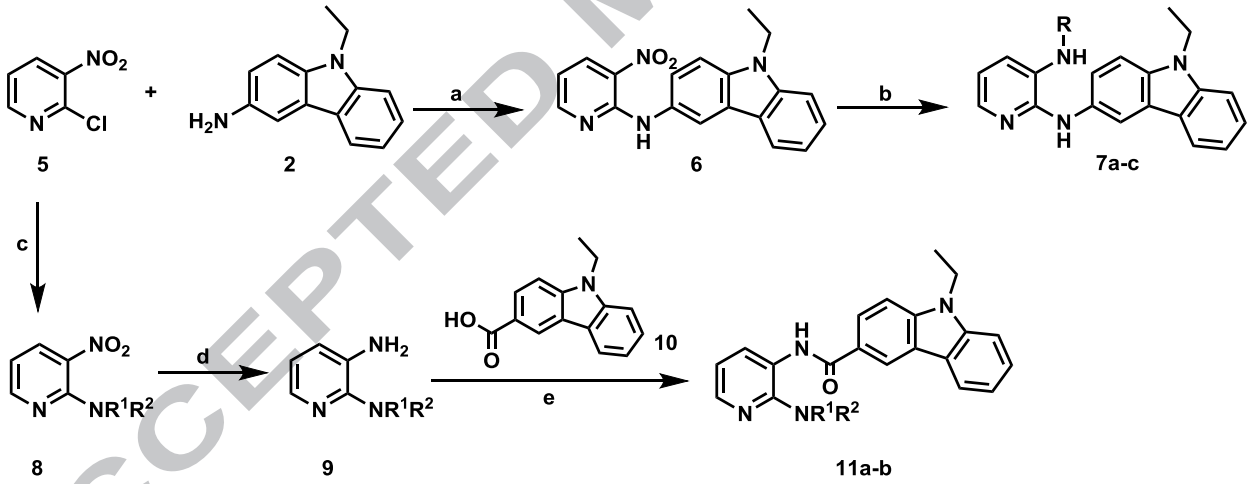
| Cmpd | R = | $GI_{50}$ (µM)[a] | | Migration (%)[b,c] |
|---|---|---|---|---|
| | | MCF-7 | MDA-MB-231 | |
| **3a** |  | >50 | >50 | 93 ±11.67 |
| **3b** |  | >50 | >50 | **68 ±10.54** |
| **3c** |  | >50 | >50 | 99 ±1.89 |
| **3d** |  | >50 | 19 | **76 ±7.87** |
| **4a** |  | 28 | >50 | 99 ±1.28 |
| **4b** |  | >50 | >50 | 95 ±9.80 |
| **4c** |  | 27 | >50 | 93 ±2.01 |
| **4d** |  | 13 | 18 | 91 ±14.10 |
| **4e** |  | 14 | 18 | 99 ±4.12 |
| **4f** |  | >50 | >50 | 92 ±8.55 |
| **EHop-016** | | 14 | 15 | **67 ±7.55** |

[a] $GI_{50}$ = compound concentration required to inhibit MDA-MB-231 proliferation by 50% after 24 h treatment. Values are expressed as the mean of triplicate experiments, and standard deviation (SD) are <10%.

[b] After 24 h, MDA-MB-231 cellular migration was determined by measuring the distance traveled from the edge of the scratch toward the center of the scratch, relative to control.
[c] Percent relative migration values at 10 μM are the average of three independent experiments.

### *2.2 Ortho diamino-substituted EHop-016 derivatives*

The compounds described in the above paragraph have an amino group attached to the 2-position and a carbonyl group attached to the 3-position of the pyridine ring. To further explore the ortho-substituted pyridine series, we synthesized several derivatives in which both the 2- and the 3-position contain a nitrogen atom, either as an amine or as a reverse amide group. The synthesis of these ortho-diamino-substituted pyridine derivatives **7a-c** and **11a-b** is represented in scheme 2. Compounds **7a-c** were synthesized via reaction of 2-chloro-5-nitropyridine **5** with aminocarbazole **2** (scheme 2 a,b). Reduction of the nitro group of **6**, followed by acylation or alkylation provided compounds **7a-b** and **7c** respectively. On the other hand, reaction of **5** with aliphatic amines and nitro group reduction provided intermediate **9**. Amide coupling with carbazole carboxylic acid **10** provided compounds **11a-b** (scheme 2 c,d,e). Their biological activities are summarized in table 2.

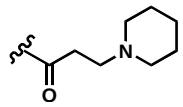
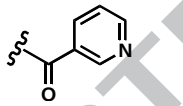
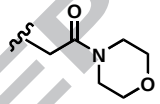
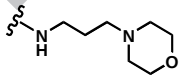
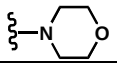



**Scheme 2.** General synthetic routes to 2,3-diamino-substituted pyridines **7a-c** and **11a-b**. Reagents and conditions: (a) THF, $Et_3N$, reflux, 2 h; (b) (i) $SnCl_2$, HCl, EtOH, reflux, (ii) RCOCl, HOBt, EDAC, DMF, $Et_3N$, rt (for **7a-b**) or R-Cl, THF, $Et_3N$, reflux (for **7c**); (c) R = $HNR^1R^2$, THF, $Et_3N$, reflux; (d) $SnCl_2$, HCl, EtOH, reflux; (e) **10**, HOBt, EDAC, DMF, $Et_3N$, rt (for **11a-b**).

Among the five compounds synthesized, three compounds (**7a-b** and **11a**) exhibited moderate anti-proliferative activity (table 2). In MDA-MB-231 cancer cells, no growth inhibition was observed for compounds **7c** and **11b** at concentrations ≤ 50 μM. Compound **11a** contains the same structural elements as compound **3d**, but reverses the amide bond, which results in a slightly increased cytotoxicity towards

MCF-7, and reduced anti-migratory activity. Similarly, compound **11b** reverses the amide bond of compound **3b**, and while both of these compounds do not inhibit growth of MCF-7 and MDA-MB-231 cell lines at a concentration ≤ 50 μM, **11b** inhibits migration of MDA-MB-231 cells by 34% compared with 32% for compound **3b**. In compound **11a**, a propylene group is inserted between the morpholine and pyridine rings of **11b**. Equivalent to the effects observed between compounds **3d** and **3b**, this modification leads to increased growth inhibitory and reduced migration inhibitory activity. Compound **7b** with an aromatic amide bond, and compound **7c** with an aliphatic group at the 3-position have moderate anti-migratory activity. From the compounds presented, **11b** is the most active inhibitor of migration so far, while it has no demonstrated growth inhibitory activity at concentrations ≤ 50 μM.

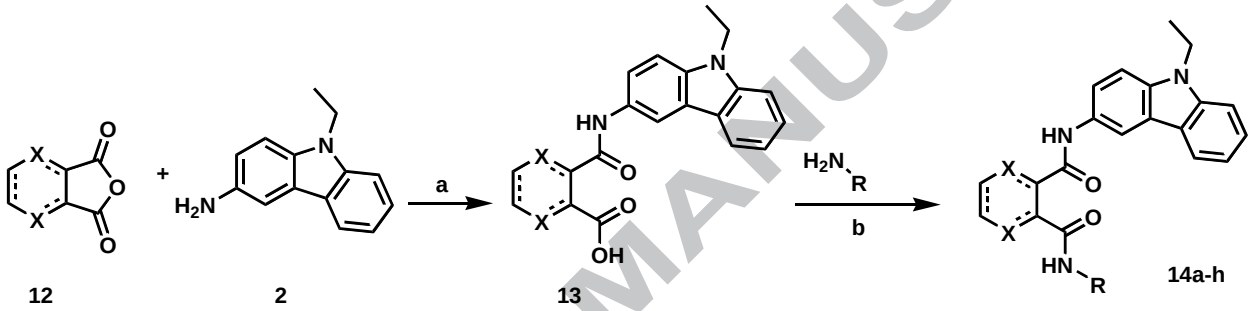
**Table 2.** Growth inhibition and anti-migration activity for compounds **7a-c** and **11a-b** on MCF-7 and MDA-MB-231 cell lines.

| **Cmpd** | **R, $R^1R^2$ =** | **$GI_{50}$ (μM)[a]** | | **Migration (%)[b,c]** |
|---|---|---|---|---|
| | | MCF-7 | MDA-MB-231 | |
| **7a** |  | 15 | 23 | 99 ±4.29 |
| **7b** |  | 41 | 31 | 81 ±11.12 |
| **7c** |  | 23 | >50 | 81 ±8.27 |
| **11a** |  | 32 | 39 | 89 ±10.10 |
| **11b** |  | >50 | >50 | **66 ±7.43** |

[a] $GI_{50}$ = compound concentration required to inhibit MDA-MB-231 proliferation by 50% after 24 h treatment. Values are expressed as the mean of triplicate experiments, and standard deviation (SD) are <10%.
[b] After 24 h, MDA-MB-231 cellular migration was determined by measuring the distance traveled from the edge of the scratch toward the center of the scratch, relative to control.
[c] Percent relative migration values at 10 μM are the average of three independent experiments.

### *2.3 Ortho dicarboxamide-substituted EHop-016 derivatives*

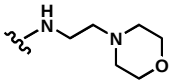
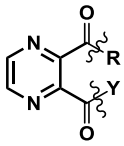
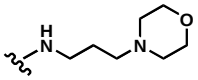
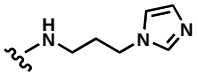
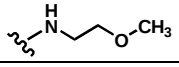
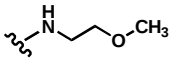
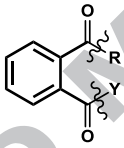
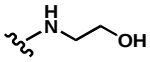
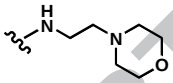
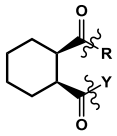
In the final series investigated, compounds with ortho-dicarboxamide containing the carbazole group that was strongly correlated with Rac1 inhibitory activity were synthesized. This was accomplished by replacing the core pyrimidine group of EHop-016 with either a benzene, pyrazine or cyclohexane group. Compounds **14a-h** were synthesized via ring-opening reaction of the corresponding cyclic anhydride building blocks with 3-amino-9-ethylcarbazole **2** in yields of 48 to 78% (scheme 3). The resulting ortho-amidocarboxylic acids **13** were reacted in an amide coupling reaction with a variety of amines in order to obtain the ortho-diamide products **14a-h** in yields of 43 to 62%. The biological activities of the compounds synthesized are summarized in table 3.




**Scheme 3.** General synthetic route to ortho-diamide derivatives **14a-h**. Reagents and conditions: (a) THF, rt 16 h; (b) HOBt, EDC, THF, rt, 16 h.

*In vitro* analysis demonstrated that the ortho-dicarboxamide derivatives did not show growth inhibition in MCF-7 and MDA-MB-231 cell lines (table 3). In addition, after 24 h of treatment at 10 μM using the wound-healing assay in the MDA-MB-231 cell line, no migration inhibition could be observed. Hence, the absence of any activity of these latter compounds, together with the fact that several compounds described in the previous paragraphs show promising activity, demonstrates that there are clear Structure Activity Relationships.

**Table 3.** Growth inhibition and anti-migration activity for compounds **14a-h** on MCF-7 and MDA-MB-231 cell lines.

| Cmpd | R = | | $GI_{50}$ (μM)[a] | | Migration (%)[b,c] |
|---|---|---|---|---|---|
| | | | MCF-7 | MDA-MB-231 | |
| **14a** |  |  | >50 | >50 | 91 ±5.23 |
| **14b** |  | | >50 | >50 | 94 ±5.87 |
| **14c** |  | | >50 | >50 | 99 ±4.50 |
| **14d** |  | | >50 | >50 | 100 ±5.20 |
| **14e** |  |  | >50 | >50 | 99 ±2.57 |
| **14f** |  | | >50 | >50 | 98 ±1.83 |
| **14g** |  |  | >50 | >50 | 99 ±1.63 |
| **14h** |  | | >50 | >50 | 100 ±0.56 |

**Y** = *N*-(9-ethyl-9H-carbazol-3-yl)

[a] $GI_{50}$ = compound concentration required to inhibit MDA-MB-231 proliferation by 50% after 24 h treatment. Values are expressed as the mean of triplicate experiments, and standard deviation (SD) are <10%.

[b] After 24 h, MDA-MB-231 cellular migration was determined by measuring the distance traveled from the edge of the scratch toward the center of the scratch, relative to control.

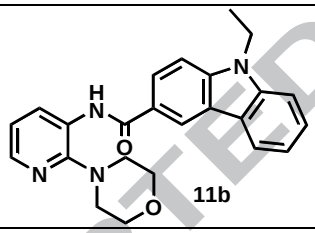
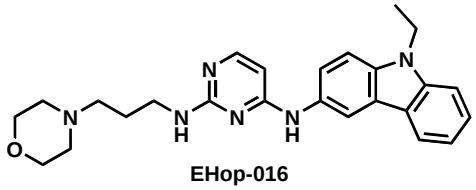
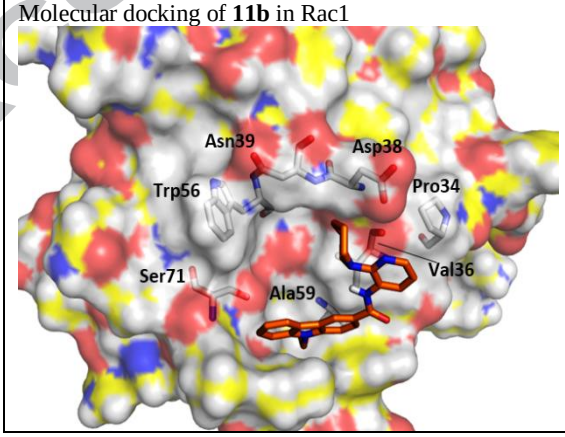
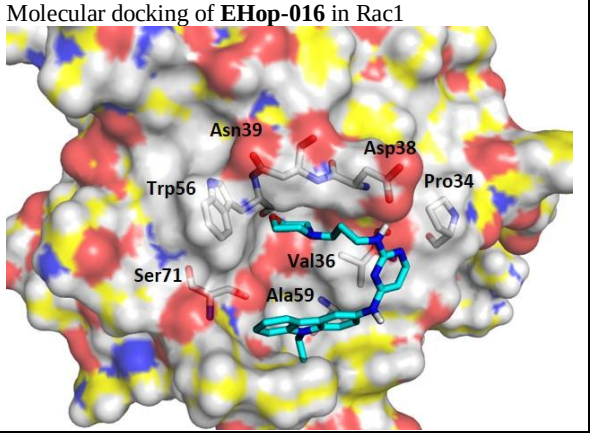
[c] Percent relative migration values at 10 μM are the average of three independent experiments.

### *2.4 Evaluation of compound **11b***

From all synthesized compounds, **11b** was the most potent inhibitor of migration, while at the same time it did not show growth inhibitory effects. For this reason, this compound was selected to determine Rac1 inhibitory activity via an ELISA-based Rac activity pulldown assay, and comparison of its

properties with EHop-016 are summarized in table 4. At 250 nM, **11b** inhibits Rac activation by 55% in both MDA-MB-231 and MDA-MB-435 cancer cells. Compared with EHop-016, with an $IC_{50}$ = 1.1 µM, **11b** is approximately 4X more potent as a Rac1 inhibitor. In addition, while EHop-016 significantly inhibits growth of all three tested cell lines at ~15 µM, including the non-tumoral mammary epithelial MCF-10A cell line, **11b** does not affect the viability at concentrations of ~30-50 µM. Molecular docking of **11b** in Rac1, closely mimics the docking of EHop-016 in the expected U-bent conformation, with the carbazole group occupying the hydrophobic pocket created by Val36 and Ala59. The central pyridine ring of 11b is in a similar position of the pyrimidine core of EHop-016, and together with the morpholine group is in close contact with the N-H group of the peptide bond between residues Val36 and Asp38. In summary, compound **11b** provides an improved Rac1 inhibitor with increased potency and reduced toxicity, and promises to be a potentially useful molecular probe for the study of migration inhibition and Rac activity.

**Table 4.** Comparison of the activities of compound **11b** (A) with **EHop-016** (B).

| A | B |
|---|---|
|  11b |  EHop-016 |
| MDA-MB-231: $GI_{50}$ > 50 µM | MDA-MB-231: $GI_{50}$ = 15 µM |
| MCF-7: $GI_{50}$ > 50 µM | MCF-7: $GI_{50}$ = 14 µM |
| MCF-10A: No growth inhibition up to 36 µM[a] | MCF-10A: 50% growth inhibition at 10 µM[20] |
| **Rac1 inhibition**<br>MDA-MB-435: 55% inhibition at 250 nM[b]<br>MDA-MB-231: 55% inhibition at 250 nM[b] | **Rac1 inhibition**<br>MDA-MB-435: $IC_{50}$ = 1.1 µM[20]<br>MDA-MB-231: $IC_{50}$ = 3 µM[20] |
| Molecular docking of **11b** in Rac1 | Molecular docking of **EHop-016** in Rac1 |
|   |   |

[a] Supplemental Figure S4
[b] Supplemental Figure S3

## 3. Conclusions

In this research, we explored the Structure Activity Relationships of novel derivatives of the lead Rac1 inhibitor EHop-016. The new compounds maintain the carbazole group of EHop-016 that previously was found to significantly contribute to Rac inhibition as a key structural feature. However, its central pyrimidine core was replaced with isosteric groups that allowed the carbazole group to be positioned in an ortho-relation with a wide diversity of substituents. Molecular dockings had suggested that ortho-substituted compounds would more easily adapt the U-shaped conformation that was calculated for the binding of EHop-016 to Rac1. The activity profile of the compounds was quite diverse. For example, compounds **4d** and **4e** inhibited growth at ~15 μM, but did not significantly inhibit migration. On the other hand, compounds **3b** and **11b** inhibited migration at 10 μM, comparable to EHop-016, but did not inhibit growth at concentrations ≤ 50 μM in the cell lines tested. As the most potent inhibitor of migration, activity of **11b** was further investigated and found to inhibit Rac1 by 55% at a concentration of 250 nM. Thus, while **11b** is a 4X more potent inhibitor of Rac1 than EHop-016, it has reduced cellular toxicity. This will be beneficial for its use as a molecular probe for the study of Rac activity, or potentially as an anti-metastatic cancer drug. Further studies of the mechanism of action are needed to fully analyze the potential of this novel compound.

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**Supplementary data**

Supplementary data associated with this article can be found in the online version.

**References and notes**


1. Hunter, K.W.; Crawford, N.P.; Alsarraj, J. *Breast Cancer Research: BCR*. **2008**, 10(Suppl 1): S2. doi:10.1186/bcr1988.
2. Chen, W.T. *J. Exp. Zool*. **1989**, 251, 167.
3. Hall, A. *Biochem. Soc. Trans*. **2005**, *33*, 891.
4. (a) Lin, M.; van Golen, K.L. *Breast Cancer Res. Treat*. **2004**, *84*, 49. (b) Kleer, C.G.; Griffith, K.A.; Sabel, M.S.; Gallagher, G.; van Golen, K.L.; Wu, Z.F.; Merajver, S.D. *Breast Cancer Res. Treat.* **2005**, *93*, 101.
5. Vega, F.M.; Ridley, A.J. *FEBS Lett*. **2008**, *582*, 2093.
6. Głuszyńska, A. *Eur. J. Med. Chem.* **2015**, *94*, 405.
7. (a) Guillonneau, C.; Pierre, A.; Charton, Y.; Guilbard, N.; Berthier, L.K.; Leonce, S.; Michael, A.; Bisagni, E.; Atassi, G. *J. Med. Chem.* **1999**, *42*, 2191. (b) Saturnino, C.; Iacopetta, D.; Sinicropi, M. S.; Rosano, C.; Caruso, A.; Caporale, A.; Marra, N.; Marengo, B.; Pronzato, M. A.; Parisi, O. I.; Longo, P.; Ricciarelli, R. Molecules **2014**, *19*, 9307.
8. Bandgar, B.P.; Adsul, L.K.; Chavan, H.V.; Jalde, S.S.; Shringare, S.N.; Shaikh, R.; Meshram, R.J.; Gacche, R.N.; Masand, V. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5839.
9. Biamonte, M.A.; Wanner, J.; Roch, K.G.L. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2829.
10. (a) Caruso, A.; Chiret, A.S.V.; Lancelot, J.C.; Sinicropi, M.S.; Garofalo, A.; Rault, S. *Molecules* **2008**, *13*, 1312. (b) Caruso, A.; Chimento, A.; El-Kashef, H.; Lancelot, J.-C.; Panno, A.; Pezzi, V.; Saturnino, C.; Sinicropi, M. S.; Sirianni, R.; Rault, S. *Journal of Enzyme Inhibition and Medicinal Chemistry* **2012**, 27(4), 609. (c) Sinicropi, M. S.; Lappano, R.; Caruso, A., Santolla, M. F.; Pisano, A., Rosano, C.; Capasso, A.; Panno, A.; Lancelot, J. C.; Rault, S.; Maggiolini, M. *Current Topics in Medicinal Chemistry*, **2015**. *15*(11), 1035. (d) Saturnino, C.; Caruso, A.; Longo, P.; Capasso. A.; Pingitore, A.; Caroleo, M.C.; Cione, E.; Perri, M.; Nicolo, F.; Nardo, V.M.; Scolaro, L.M.; Sinicropi. M.S.; Plutino. M.R.; El-Kashef, H. *Curr Top Med Chem.* **2015**

*15*(11), 973. (e) Caruso, A.; Lancelot, J. C.; El-Kashef, H.; Sinicropi. M.S.; Legacy, R.; Lesnarda, A.; Rault, S. Tetrahedron **2009**, *65*, 10400. (f) D. Iacopetta, A.; Carocci, M. S.; Sinicropi, A.; Catalano, G.; Lentini, J.; Ceramella, R.; Curcio, M. C. Caroleo, *Chem Med Chem* **2017**, *12*, 381.

11. (a) Chakrabarty, M.; Ghosh, N.; Harigaya, Y. *Tetrahedron Lett.* **2004**, *45*, 4955. (b) Caruso, A.; Sinicropi. M.S.; Lancelot, J. C.; El-Kashef, H.; Saturnino, C.; Aubert, G.; Lesnard, A.; Cresteil, T.; Dallemagne, P.; Rault, S. *Bioorg Med Chem Lett* **2014**, *24*, 467. (c) Iacopetta, D.; Rosano, C.; Puoci, F.; Parisi, O.; Saturnino, C.; Caruso, A.; Longo, P.; Ceramella, J.; Malzert-Fréon, A.; Dallemagne, P.; Rault, S.; Sinicropi, M. *Eur J Pharm Sci.* **2017**, *96*, 263. (d) Caruso, A.; Iacopetta, D.; Puoci, F.; Cappello, A. R.; Saturnino, C.; Sinicropi, M. S. *Mini Rev Med Chem*. **2016**, *16*(8), 630. (e) Saturnino, C.; Palladino, C.; Napoli, M.; Sinicropi, M. S.; Botta, A.; Sala, M.; Carcereri de Prati, A.; Novellino, E.; Suzuki, H. *European Journal of Medicinal Chemistry* **2013**, *60* 112. (f) Rizza, P.; Pellegrino, M.; Caruso, A.; Iacopetta, D.; Sinicropi, M. S.; Rault, S.; Lancelot, J. C.; El-Kashef, H.; Lesnard, A.; Rochais, C.; Dallemagne, P.; Saturnino, C.; Giordano, F.; Catalano, S.; Ando, S. *European Journal of Medicinal Chemistry* **2016**, 107, 275.
12. Issa, S.; Walchshofer, N.; Kassab, I.; Termoss, H.; Chamat, S.; Geahchan, A.; Bouaziz, Z. *Eur. J. Med. Chem.* **2010**, *45*, 2567.
13. Danish, A.I.; Prasad, K.J.R. *Indian J. Heterocycl. Chem.* **2006**, *14*, 19.
14. Indumati, T.; Fronczek, F.R.; Prasad, K.J.R. *J. Mol. Struct.* **2012**, *1016*, 134.
15. Kantevari, S.; Yempala, T.; Surineni, G.; Sridhar, B.; Sriram, D. *Eur. J. Med. Chem.* **2011**, *46*, 4827.
16. Bashir, M.; Bano, A.; Subhan-Ijaz, A.; Ahmad-Chaudary, B. *Molecules* **2015**, *20*, 13496.
17. Wang, Y.M.; Hu, L.X.; Liu, Z.M.; You, X.F.; Zhang, S.H.; Qu, J.R.; Li, Z.R.; Li, Y.; Kong, W.J.; He, H.W.; Shao, R.G.; Zhang, L.R.; Peng, Z.G.; Boykin, D.W.; Jiang, J.D. *Clin. Cancer Res.* **2008**, *14*, 6218.

18. Liu, C.H.; Lin, C.; Tsai, K.J.; Chuang, Y.C.; Huang, Y.L.; Lee, T.H.; Huang, L.J.; Chan, H.C. *Oncol. Rep.*, **2013**, *29*, 1501.

19. Yoon, S.; Kim, J.H.; Lee, Y.J.; Ahn, M.Y.; Choi, G.; Kim, W.K.; Yang, Z.; Lee, H.J.; Moon, H.R.; Kim, H.S. *Eur. J. Pharmacol.* **2012**, *697*, 24.

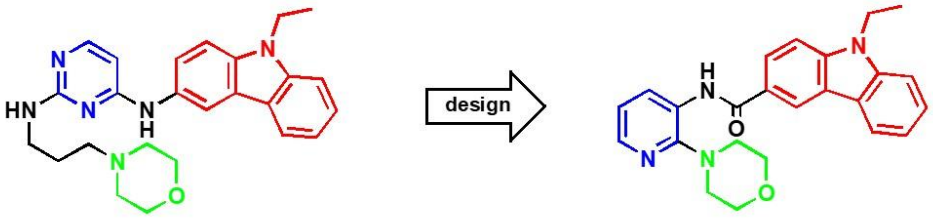
20. Montalvo-Ortiz, B. L.; Castillo-Pichardo, L.; Hernández, E.; Humphries-Bickley, T.; De La Mota-Peynado, A.; Cubano, L. A.; Vlaar, C. P.; Dharmawardhane, S. J. Biol. Chem. **2012**, *287*, 13228.

21. Castillo-Pichardo, L.; Humphries-Bickley, T.; de la Parra, C.; Forestier-Roman, I.; Martínez-Ferrer, M.; Hernández, E., et al. Transl. Oncol. **2014**, *7*, 546.

22. Orellana, E. A.; Kasinski, A. L. *Bio Protoc*. **2016** 6(21), 1984.

23. Liang, C-C.; Park, A.Y.; Guan, J-L. Nature Protoc. **2007**, *2*, 329.

24. Note: A U.S. Provisional Patent Application No.: 62/577,305 (Filing Date: October 26, 2017) has been submitted.




**EHop-016**, 1.1 mM

**Rac1 inhibition**

**11b**, 250 nM

25.