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

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

[a] Department of Pharmaceutical Sciences, School of Pharmacy, University of Puerto Rico, San Juan, PR 00936, United States
[b] Department of Pathology and Laboratory Medicine, School of Medicine, Universidad Central del Caribe, Bayamon, PR 00960, United States

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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 anti-cancer 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.

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.

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 Fig. 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

* Corresponding author.
E-mail address: eliud.hernandez@upr.edu (E. Hernández).

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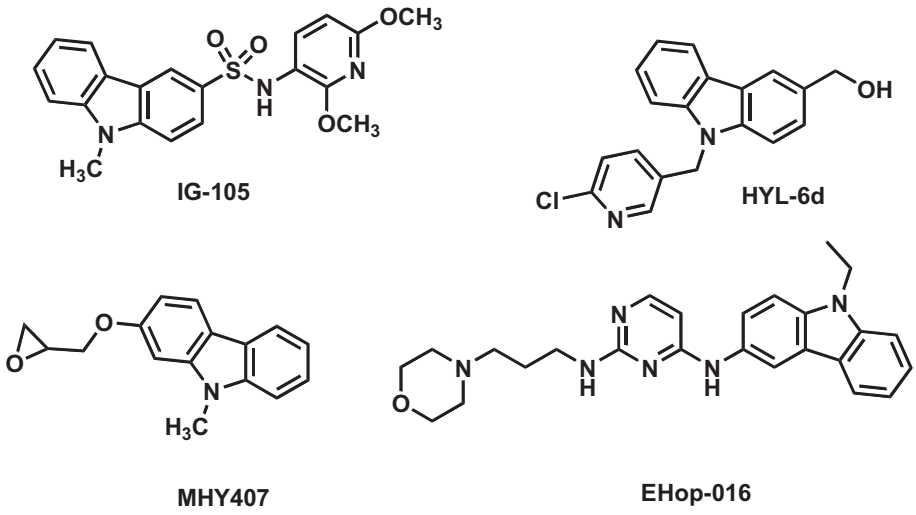

Fig. 1. Structure of representative anti-cancer carbazole derivatives.

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 as inhibitor of tumor growth and metastasis *in vivo* was demonstrated in a 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 (Fig. 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 1–3.

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 Fig. 2. It can be observed that in the control, after 12 h, wound healing is progressing considerably, and after 24 h, the wound is basically healed. In contrast, in the presence of **3b** or **11b**, both after 12 and 24 h, 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 1–3.[24]

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.

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