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Original Research Article

Design, Synthesis and Evaluation of N-Aryl Carboxamide Derivatives as Potential Anti-Proliferative Effect on the Pulmonary Artery Smooth Muscle Cells

Khalid Rhazri[1], Mohamed Izikki[2*], Jamila Bouali[1], Salha Hamri[1], Jouha Jabrane[1], Youssef Hajbi[1], Abderrafia Hafid[1], Saadia Eddahibi[2], Mostafa Khouili[1]

[1]Laboratoire de Chimie Organique & Analytique, Université Sultan Moulay Slimane, Faculté des Sciences et Techniques, BP 523, 23000 Béni-Mellal, Morocco.

[2]INSERM, U999, Centre chirurgical Marie Lannelongue, 133 Avenue de résistance, 92350 Le Plessis Robinson, France.

* **Corresponding Author:** Mohamed Izikki, INSERM U999/ Marie Lannelongue hospital 133, Avenue de la Résistance / 92350 - Le Plessis Robinson - FRANCE

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ABSTRACT

A new series of compounds, cinnamamide's and 2-hydroxy-*N*-arylbenzamide's derivatives, were synthesized by condensation reaction of cinnamic acid chlorides and salicylic acid chlorides with amino-aryl's derivatives. The chemical structure of these compounds was confirmed by means ^{1}H NMR and ^{13}C NMR. All of the compounds were assayed for anti-proliferative effect on isolated pulmonary arterial smooth muscle cells (PA-SMCs) by BrdU incorporation. We found that compounds: 5, 6, 9-13 and 16, showed marked inhibition of PA-SMCs proliferation. Structure–activity relationship analysis showed that compounds benzamide's derivatives have better inhibitory effects than the others.

Keyword: Cinnamide; *N*-Aryl-Carboxamide; Pulmonary arterial hypertension; Pulmonary arterial smooth muscle cells; Heteroarylamines

INTRODUCTION

Pulmonary Arterial Hypertension (PAH) is diagnosed by an elevation in mean pulmonary arterial pressure above 25 mmHg at rest or 30 mmHg with exercise [1]. PAH is a pulmonary selective vascular remodeling disease in which cells within the vessel wall, including pulmonary artery smooth muscle cells (PA-SMCs), are characterized by excessive proliferation and an impaired apoptosis due to dysregulation of multiple cellular signaling mechanisms [2]. The current therapeutic approaches for the treatment of pulmonary hypertension (PH)

mainly provide symptomatic relief in addition to some improvement of prognosis. Indeed, pulmonary artery smooth muscle cells in idiopathic pulmonary hypertension have some tumor-like characteristics as excessive proliferation and resistance to apoptosis and many drugs initially developed as a treatment for cancers have a beneficial effect for the treatment of PAH [3,4].

The synthesis of novel series of cinnamamide derivatives and benzamide derivatives, from of cinnamic acid and salicylic acid respectively, experienced a revival of interest in the recent years due to their various biological and / or pharmacological properties [5-8]. Indeed, cinnamic acid and salicylic acid exert an antitumor activity against lung adenocarcinoma cells [9] and colon carcinoma cells [10, 11]. Phuwapraisirisan et *al*. [12], have described a series of cinnamide's derivatives (I) isolated from the leaves Aegle: anhydromarmeline aegelinosides, which are inhibitors of alpha-glucosidase. Among the derivatives having the functional group α,β-unsaturated carboxamides of the compounds (II) [13] having similar effects with neuroprotective particularly edaravone and other derivatives (III) acting on the reduction of tumor growth in xenograft models of human tumors have been reported [10] (Fig. 1):

The purpose of this study was to investigate the synthesis of some new derivatives of cinnamide and benzamide, and the effects of these compounds on the growth of PA-SMCs that occurs in response to incubation with 10% Fetal Calf Serum (FCS).

MATERIAL AND METHODS

Chemistry

This method was implemented punctually in cinnamide series, during the synthesis of *N*-phenylcinnamamide. Our strategy first required the preparation of the acid chloride **1A** by reaction of cinnamic acid **1** with thionyl chloride ($SOCl_2$). The reaction is conducted without solvent, in the presence of a large excess of reagent. [13-14] After 2 hours of stirring at reflux, the unpurified acid chloride obtained after removal of the excess reagent is immediately involved in the amidation reaction. This is accomplished by slowly adding the acid chloride to a solution of the amine in anhydrous 1,2-dichloroethane, in the presence of triethylamine. Stirring at room temperature was maintained for 5 hours, then the triethylamine hydrochloride was removed by filtration. The amide purified on silica gel column is obtained with yields of about 65-96%. Prepared this way, the different benzamides (**7-15**) have also been isolated in good yields.

Chemistry general

Melting points were taking for samples in capillary tubes with an electro-thermal apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR were recorded on a Bruker Avance DPX250 spectrometer (300 MHz ^{1}H, 75.5 MHz ^{13}C) using tetramethylsilane as the internal standard, chemical shifts were reported in parts per million (ppm, δ units). Coupling constants were reported in units of hertz (Hz). Flash chromatography was performed on silica gel 60 (40–63 mesh). Thin layer chromatography (TLC) was carried out on Merck silica gel 60F254 pre-coated plates. Visualization was made with ultraviolet light. All organic solvents were distilled immediately prior to use.

General procedure for the preparation of cinamides (3-8) [13, 14]

To a suspension of (1g; 6.75 mmol) acid cinnamic **1** in dry benzene (20 mL) and dry pyridine (0.5 mL) at 0 °C, thionyl chloride (2 mL; 13.5 mmol) was added slowly. The mixture was then vigorously stirred at 0 °C. After 2 h, the benzene and excess thionyl chloride were removed in vacuo. The crude residue was

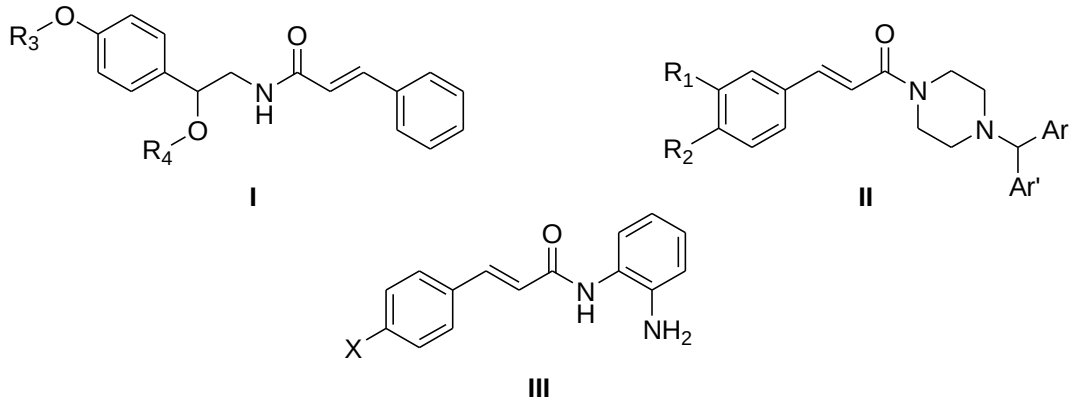


Figure 1. Functional carboxamide group α,β-unsaturated.

dissolved in anhydrous benzene (20 mL), cooled to 0°C, and 10.12 mmol of aniline derivatives were added dropwise. The reaction mixture was stirred at room temperature during one night. The solvent was removed; the mixture was quenched with a solution of thiosulfate de sodium and extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, evaporated, and purified by flash chromatography (eluant: CH_2Cl_2).

Data for compounds

***N*-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl) cinnamamide (3)**

Following the general procedure, compound **3** was obtained as a brown solid in 80% yield, mp: 200-202 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.72 (d, 1H, *J* = 16 Hz, CH=CH); 7.50 (d, 1H, *J* = 8.8 Hz, H_7); 7.38-7.35 (m, 5H, Ph); 7.00 (s, 1H, H_5); 6.81 (d, 1H, *J* =
8.8 Hz, H_8); 6.52 (d, 1H, J = 16 Hz, CH=CH); 4.24 (m, 4H, CH_2O). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm), 64.3 (CH2 (×2), C-2, C-3); 105.5 (CH, C-8); 113.8 (CH, C-6); 115.2 (CH, C-8); 118.9 (CH=); 128.0 (CH, Ph); 126.4 (2CH, Ph); 128.1 (C-N); 128.7 (2CH, Ph); 135.2 (**C**-CH=); 142.3 (C-O); 144.0 (CH=); 146.9 (C-O); 166.7 (CO). IR (KBr) cm^{-1}: 3044 (NH); 1654 (C=O); 1548 (CN); 1500 (CC); 1217 (Ar-O); 1066 (C-O). ESI-HRMS: m/z [M + H] calcd for C17H16NO3: 282.1130, found 282.1125.

***N*-phenylcinnamamide (4)**

Following the general procedure, compound **4** was obtained as a white solid in 94% yield, mp: 159-161 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.74 (d, 1H, *J* = 15.4 Hz, CH=CH); 7.61-7.37 (m, 10H, Ph); 6.56 (d, 1H, *J* = 15.4 Hz, CH=CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm): 120.9 (CH=); 124.5 (C-Ph); 128.1-129.9 (CH, Ph); 138.0 (C-N); 142.5 (CH=); 166.4 (CO). IR (KBr) cm^{-1}: 3035 (NH), 1660 (C=O), 1594 (CC), 1542 (CN). ESI-HRMS: m/z [M + H] calcd for C15H14NO: 224.1075, found 224.1082.

***N*-(4-chlorophenyl) cinnamamide (5)**

Following the general procedure, compound **5** was obtained as an orange solid in 76% yield, mp: 140-142 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.62-7.50 (m, 3H, Ar-H and O=C-C=CH); 7.32-7.12 (m, 7H, Ar-H); 6.60 (d, 1H, *J* = 16.8 Hz, O=C-CH=C). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm), 120.4 (CH=); 121.2 (C-Ph); 128.1-128.9-129.1-129.4 (5CH, Ph); 130.2 (C-Cl); 134.4 (C-N); 142.9 (CH=); 163.0 (CO). IR (KBr) cm^{-1}: 2922 (NH); 1659 (C=O); 1590 (CC); 1514 (CN); 821 (Ar-Cl). ESI-HRMS: m/z [M + H] calcd for C15H13ClNO: 258.0686, found 258.0677.

_N_-(4-hydroxyphenyl) cinnamamide (6)

Following the general procedure, compound **6** was obtained as a yellow solid in 72% yield, mp: 144-146 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.90 (d, 1H, *J* = 16 Hz, CH=CH); 7.57-7.19 (m, 9H, Ph); 6.54 (d, 1H, *J* = 16 Hz, CH=CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm), 117.1 (CH=); 122.9 (C-Ph); 127.6 ($2CH_{ortho}$); 128.0 ($2CH_{méta}$); 128.6-128.9-129.9 (5CH, Ph); 130.0 (C-C=); 130.8 (C-N); 146.8 (C-OH); 164.0 (CO). IR (KBr) cm^{-1}: 3232 (OH); 3049 (NH); 1655 (C=O); 1542 (CN); 1502 (CC); 1154 (Ar-O); 1134 (C-O). ESI-HRMS: m/z [M + Na] calcd for C15H13NNaO2: 262.0844, found 262.0846.

_N_-_o_-tolylcinnamamide (7)

Following the general procedure, compound **7** was obtained as a white solid in 93% yield, mp: 125-127 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.92 (s, 1H, NH);7.70 (d, 1H, J = 15.5 Hz, CH=CH); 7.50-7.34 (m, 5H, Ph); 7.20 (d, 2H, *J* = 7.8 Hz, H); 7.10 (d, 2H, J = 7.8 Hz, H); 6.60 (d, 1H, *J* = 15.5 Hz, CH=CH); 2.30 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm), 17.9 (CH_3); 120.9 (CH=); 123.4-124.5-126.8 ($4CH_{Ph}$); 128.0-128.8-129.0 ($5CH_{Ph}$); 130.0 (C-CH3); 130.6 (C-N); 134.6 (C-C=); 142.3 (CH=); 164.0 (CO). ESI-HRMS: m/z [M + H] calcd for C16H16NO: 238.1232, found 238.1224.

_N_-(4-methoxyphenyl) cinnamamide (8).

Following the general procedure, compound **8** was obtained as a brown solid in 96% yield, mp: 145-147 °C. ^{1}H NMR ($CDCl_3$, 300 MHz) δ (ppm): 7.72 (d, 2H, *J* = 15.5 Hz, CH=CH); 7.54 (d, 1H, *J* = 8 Hz, H); 7.38 (m, 5H, Ph); 6.88 (d, 2H, *J* = 8 Hz, H); 6.54 (d, 1H, J = 15.5 Hz, CH=CH); 3.80 (s, 3H, OCH_3). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ (ppm), 55.6 (OCH_3); 114.2 ($2CH_{ortho}$); 120.9 (CH=); 122.0 ($2CH_{meta}$); 128.3-128.9 (5CH, Ph); 129.8 (C-N); 134.7 (C-C=); 141.9 (CH=); 156.5 (C-O); 164.3 (CO). ESI-HRMS: m/z [M + H] calcd for C16H16NO2: 254.1181, found 254.1202.

General procedure for the preparation of amides (9-17)

A mixture of acid salicylic **2** (1g; 7.28 mmoL) and thionyl chloride (6.3 mL), contained in a 100 ml round-bottomed flask, was refluxed in an oil bath. After 2 h, the $SOCl_2$ was removed. The crude product was dissolved in CH_2Cl_2 (7 mL) and stirred at 0°C. At that point, a solution of appropriate amine (1.2 eq.) was added dropwise in dichloromethane (7 mL) and triethylamine (NEt_3 1.2 mL). The reaction mixture was left overnight at room temperature. After elimination of CH_2Cl_2 the reaction was purified by flash chromatography (CH_2Cl_2/ hexane).

_N_-(4-chlorophenyl)-2-hydroxybenzamide (9)

Following the general procedure, compound **9** was obtained as a white solid in 67% yield. Mp: 184-186 °C. ^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.58 (s, 1H); 10.13 (s, 1H); 6.90-7.67 (m, 8H); ^{13}C NMR (DMSO, 75.5 MHz) δ (ppm), 164.8 (C); 159.4 (C); 136.0 (C); 133.6 (CH); 129.9 (C); 129.2 (2CH); 129.14 (CH); 124.0 (2CH); 120 (C); 118.0 (CH). ESI-HRMS: m/z [M + Na] calcd for C13H10NNaO2: 270.0298, found 270.0301.

2-hydroxy-_N_-(5-oxo-1-phenyl-4,5-dihydro-1_H_-pyrazol-3-yl)benzamide (10).

Following the general procedure, compound **10** was obtained as a yellow crystal in 66 % yield. Mp: 196-198 °C. ^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.90 (s, 1H) ; 10.62 (s, 1H) ; 6.15 (s, 2H); 6.92-8.12 (m, 9H); ^{13}C NMR (DMSO, 75.5 MHz) δ (ppm), 172.8 (C); 175.0 (C); 169.0 (C); 159.4 (C); 140.4 (C); 133.6 (CH); 129.0 (2CH); 128.9 (CH); 124.4 (CH); 121.6 (2CH); 121.5 (CH); 120 (C); 117.0 (CH); 39.9 (CH_2). ESI-HRMS: m/z [M + H] calcd for C16H14N3O3: 296.1035, found 296.1092.

2-hydroxy-_N_-(2-methyl-3-nitrophenyl)benzamide (11). Following the general procedure, compound **11** was obtained as a yollew solid in 68% yield Mp: 122-124 °C

.^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.89 (s, 1H); 10.50 (s, 1H); 5.52 (s, 3H); 6.79- 8.05 (m, 7H). ^{13}C NMR (DMSO, 75.5 MHz) δ (ppm), 164.8 (C); 159.4 (C); 149.3 (C); 135.7 (C); 133.6 (CH); 128.9 (CH); 127.6 (CH); 126.9 (CH); 124.7 (CH); 121.5 (CH); 120.0 (C); 116.6 (CH); 116.0 (CH); 6.6 (CH_3). ESI-HRMS: m/z [M + H] calcd for C14H13N2O4: 273.0875, found 273.0851.

2-hydroxy-*N*-(4-fluorophenyl)benzamide (12). Following the general procedure, compound **12** was obtained as a white crystal in 65% yield Mp: 169-171 °C.^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.79 (s, 1H); 10.50 (s, 1H); 6.87-7.90 (m, 9H).^{13}C NMR (DMSO, 75.5 MHz) δ (ppm), 164.8 (C); 159.4 (C); 133.6 (CH); 131.5 (C); 128.9 (CH); 123.2 (2CH); 121.5 (CH); 120 (C); 116.0 (CH); 115.7 (2CH). ESI-HRMS: m/z [M + H] calcd for C13H11FNO2: 232.0774, found 232.0781.

2-hydroxy-*N*-(4-hydroxyphenyl)benzamide (13). Following the general procedure, compound **13** was obtained as a brown solid in in71% yield Mp: 154-156 °C. ^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.89 (s, 1H); 10.39 (s, 1H); 6.97-7.97 (m, 9H). ^{13}C NMR (DMSO, 75.5 MHz) δ (ppm), 164.8 (C); 159.4 (C); 133.6 (CH); 135.9 (C); 129.0 (2CH); 128.9 (CH); 124.4 (CH); 121.6 (2CH); 121.5 (CH);120.0 (C); 116.0 (CH). ESI-HRMS: m/z [M + H] calcd for C13H12NO2: 214.0868, found 214.0822.

2-hydroxy-*N*-(4-cyanophenyl)benzamide (14). Following the general procedure, compound **14** was obtained as a brown solid in 73% yield Mp: 163-165 °C. ^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.41 (s, 1H); 10.70 (s, 1H); 6.99-7.97 (m, 8H). ^{13}C NMR (DMSO, 75.5 MHz) δ (ppm) 164.8 (C); 159.4 (C); 140.2 (C); 133.6 (CH); 132.4 (2CH); 128.9 (CH); 122.3 (2CH); 121.5 (CH); 119.9 (C); 116.0 (CH); 115.8 (C); 108.2 (C). ESI-HRMS: m/z [M + H] calcd for C14H11N2O2: 239.0821, found 239.0798.

2-hydroxy-*N*-(4-hydroxyphenyl)benzamide (15). Following the general procedure, compound **15** was obtained as a white solid in 70% yield. Mp: 154-156 °C .^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.75 (s, 1H); 10.45 (s, 1H); 10.27 (s, 1H); 7.78-6.85 (m, 8H); ^{13}C NMR (DMSO, 75.5 MHz) δ (ppm), 167.8 (C); 159.4 (C); 154.1 (C); 130.1 (C); 137.2 (CH); 131.8 (CH); 123.1 (2CH); 121.5 (CH); 120.1 (C); 118.1 (2CH); 118.0 (CH). ESI-HRMS: m/z [M + Na] calcd for C13H11NNaO3: 252.0637, found 252.0638.

2-hydroxy-*N*-(3-methylpyridin-2-yl)benzamide (16). Following the general procedure, compound **16** was obtained as a yellow solid in 56% yield Mp: 136-138 °C.^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.89 (s, 1H); 10.50 (s, 1H); 2.41 (s, 3H); 6.86- 7.99 (m, 7H). ^{13}C NMR (DMSO, 75.5 MHz) δ (ppm), 164.8 (C); 159.4 (C); 154.6 (C); 145.9 (CH); 137.5 (CH); 133.6 (CH); 128.9 (CH); 121.5 (CH);120 (C); 117.0 (C); 116.0 (CH); 112.5 (CH); 17.9 (CH_3). ESI-HRMS: m/z [M + H] calcd for C13H12N2O2: 229.0977, found 228.0945.

2-hydroxy-*N*-(4-methoxyphenyl)benzamide (17). Following the general procedure, compound **17** was obtained as a yellow solid in 74.6% yield Mp: 149-151 °C.^{1}H NMR (DMSO, 300 MHz) δ (ppm), 11.98 (s, 1H); 10.27 (s, 1H); 7.95-6.15(m, H,); 5,7 (s, 3H).^{13}C NMR (DMSO, 75.5 MHz) δ (ppm) 164.8 (C); 159.4 (C); 156.3 (C); 133.6 (CH); 128.9 (CH); 128.2 (C); 122.6 (2CH); 121.5 (CH);120.0 (C); 116.0 (CH); 114.5 (2CH); 55.9 (CH_3). ESI-HRMS: m/z [M + H] calcd for C14H14NO3: 244.0974, found 244.0985.

Ethics Statement

This study was approved by the institutional review board and the local ethics committee (Comité de Protection des Personnes, Ile-de-France VII, Le Kremlin-Bicêtre, France). Written, informed consent was given by all the patients prior to their contribution to the study. We studied lung specimens obtained from 8

patients during lobectomy or pneumonectomy for localized lung cancer. Preoperative echocardiography was performed to rule out PH, and the lung specimens were collected at a distance from the tumor foci.

Isolation, culture, and proliferation test of human PA-SMCs

PA-SMCs were isolated and cultured as previously described [22]. To identify PA‑SMCs, we examined cultured cells for expression of muscle‑specific contractile and cytoskeletal proteins, including smooth-muscle α‑actin, desmin, and vinculin. Cells were used between passages 3 and 6.

PA‑SMCs were subjected to 48 hours of growth arrest in serum-free medium and were then treated with compounds (10 µM) 1 h prior to incubation with 10% fetal calf serum (FCS) (Eurobio, Courtaboeuf, France). For each condition, the cells were incubated for 24 hours and PA-SMC proliferation was then measured by 5-bromo-2-deoxyuridine (BrdU) incorporation.

Statistical analysis

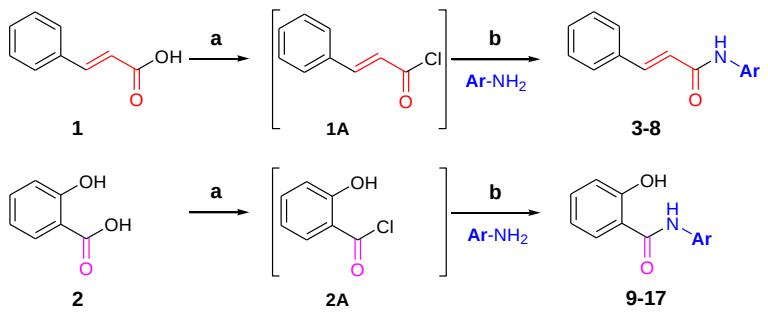
All results were reported as the mean plus or minus the SEM. For studies performed on PA-SMCs, the nonparametric Mann-Whitney test was used for comparisons between groups.

RESULTS AND DISCUSSIONS

Chemistry:

According to the procedures reported in literature, *N*-arylcarboxamide's derivatives can be synthesized by condensation of an amine with an acid, after its activation, and the great diversity of methods currently used, partly reflect the methodologies developed for the synthesis of peptides and polyamides [13-19]. During the last thirty years, many reagents have been introduced as coupling agents in order to reduce the time and temperature of the reaction and in order to afford easily removable by-products allowing a more easy purification of the crude. In this work we activated the carboxylic acid transforming it into the corresponding halides by treatment with thionyl chloride $SOCl_2$ and then effecting the reaction with the desired amine in the presence of a tertiary amine, used as scavenger of the hydrohalides acid. The procedure was applied starting from commercially available cinnamic acid and salicylic acid followed by a condensation reaction with various amino heteroaryl moiety [20, 21].

In order to investigate the HTA's activities of cinnamamides and benzamides, a series of these compounds were prepared following the above described protocol and are reported in Scheme 1.

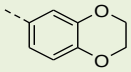
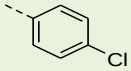
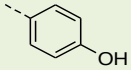
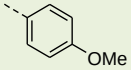


Scheme 1. Preparation of carboxamide group: a) $SOCl_2$, Reflux, 2h; b) NEt_3, CH_2Cl_2, 5h.

The acylchloride derivatives (1A) are prepared starting from cinnamic acid without further purification (1A) (scheme 1), after removal of the excess of chlorinating agent, was immediately involved in the amidation reaction with the aromatic amines 1-6. This step was

carried out by slow addition of the amine dissolved in dichloromethane under vigorous stirring at room temperature (Scheme 1). The yields, reported in table 1, range between 72% and 96%. Less satisfactory results were obtained using cinnamic acid and 3-amino-1-phenyl-1*H*-pyrazol-5(4*H*)-one for the synthesis of *N*-(5-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazol-3-yl)cinnamamide, in all the attempts only starting materials were recovered. Similarly 4-methylbenzenesulfonic acid afforded the final product only in 15% of yield. This latter result can be explained by the presence of the sulfonic acid function that is relatively unreactive in basic medium, Table 1.

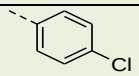
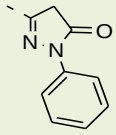
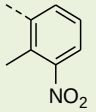
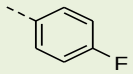
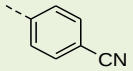
Table 1: Coupling reaction with cinnamic acid and various heteroarylamines (3-6)

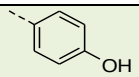
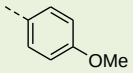
Entry	Ar	Product	Yield (%)[a]
1		3	80
2		4	94
3		5	76
4		6	72
5		7	93
6		8	96

[a] Yield of pure isolated product.

In order to overcome the instability of the intermediate (**1A**) we move to the acyl chloride of the salycilic acid (**2A**). A series of amides (**9-17**) were prepared in a similar way by the condensation of 2-hydroxybenzoyl chloride (**2A**) with various amines in good to excellent yields (Scheme 1, Table 2).

Table 2: Coupling reaction with salicylic acid and various hetero arylamines (9-17)

Entry	Ar	Product	Yield (%)[a]
1		9	67
2		10	80
3		11	70
4		12	65
5		13	71
6		14	73

7		15	70
8		16	66
9		17	75

[a] Yield of pure isolated product.

All new synthesized compounds were purified, analyzed, and their structures were determined based on the spectral data of IR, NMR (^{1}H and ^{13}C), mass spectroscopy and High resolution mass spectrometry (HRMS). In general, IR spectra showed the C=O peak at 1625-1665 cm-1, the NH stretching vibrations at 3220-3290 cm-1. The signals of the protons of the analysis of nuclear magnetic resonance spectra (^{1}H-NMR) were determined and checked on the basis of their chemical shifts, multiplicities and their coupling constants.

Effect of Some New Derivatives of Cinnamamide and 2-Hydroxy-*N*-arylbenzamide on PA-SMCs proliferation

We used BrdU incorporation assays, to investigate the ability of cinnamamide and 2-hydroxy-*N*-arylbenzamide's derivatives to inhibit cell proliferation. The cultures of human PA-SMCs in medium supplemented with 10% FCS were treated with some new derivatives of cinnamide and benzamide at a final concentration of 10 µM for 24 hours.

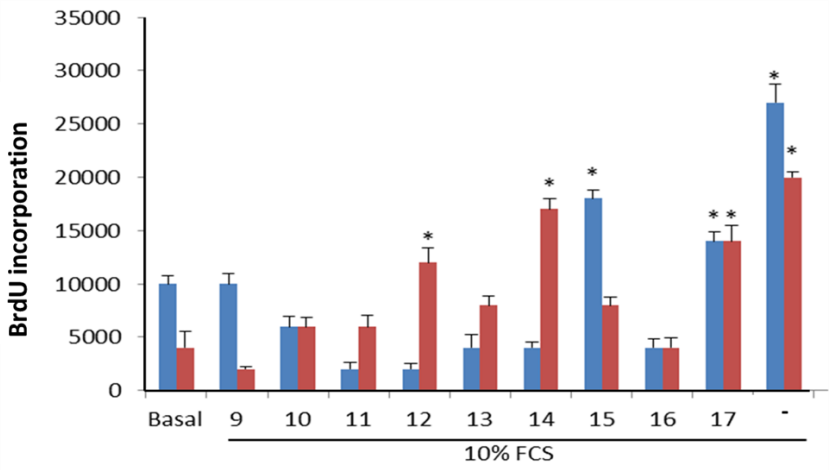


Figure 2. *The effect of new derivatives of cinnamide on fetal calf serum-induced pulmonary-artery smooth muscle cell proliferation. BrdU incorporation in human pulmonary-artery smooth muscle cells in response to 10% fetal calf serum (FCS) in the presence or absence of new derivatives of cinnamide. These compounds were added 1 hour before FCS addition. n=5; *P<0.0001.*

The observed degree of BrdU incorporation indicated an inhibition of PA-SMC's growth due to our compounds (Fig. 2). Indeed, the addition of 10% FCS to the medium increased BrdU incorporation, but these effects were completely abolished when the cells were pretreated with the following compounds: 5, 6, 9, 10, 11, 12, 13, and 16. The doses of cinnamide's and benzamide's derivatives applied had no toxic effect; indeed, cell viability and apoptosis were similar in the cells treated

with these compounds and those incubated with the vehicle alone.

As shown in figure 2, cinnamide's derivatives 5, 6 and benzamide's derivatives 9, 10, 11, 12, 13, and 16 exhibit an excellent antiproliferative effect on PA-SMCs growth under the same conditions. This indicated that benzamide's derivatives had a better antiproliferative effect than cinnamide's derivatives. Indeed, all these products possess a phenol function present on the salicylic acid's derivative and a carboxamide function (-NH-C=O) linking the phenol with various aryl and heteroaryl. Concerning benzamide's derivatives, compounds 9-13 and 16 (NH-C=O) have a good effect, whereas no anti-proliferative effects were shown by compounds substituted at the aromatic nucleus by groupements: methoxy (MeO-) (compound 17), nitrile (-CN) (compound 14) and phenol (-OH) (compound 15). On the other hand, benzamide derivatives, compounds 5 and 6 (cinnamamide C=C-C=O) had a marked antiproliferative effect on PA-SMCs' proliferation (presence of halogen or phenol group), whereas no anti-proliferative effects were shown with the presence of the methyl group (Compound 7), methoxy group (compound 8), unsubstituted phenyl group (Compound 4) and the heteroaryl group (Compound 3).

CONCLUSION

Results of the study have provided information for the anti-proliferative effect of these new derivatives cinnamamide and 2-hydroxy-*N*-arylbenzamide on isolated pulmonary arterial smooth muscle cells (PA-SMCs) by BrdU incorporation.

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CONFLICT OF INTEREST STATEMENT

None Declared

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