Journal of Enzyme Inhibition and Medicinal Chemistry, 2009; 24(5): 1148–1153

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RESEARCH ARTICLE

Synthesis, inhibition of NO production and antiproliferative activities of some indole derivatives

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Abstract
The synthesis and the biological evaluation of pyrano[3,2-e]indoles and their reaction intermediates are described. The compounds prepared were evaluated for their inhibition of NO production, antioxidant activity and also for their ability to inhibit *in vitro* the growth of four human tumor cell lines: large lung carcinoma (COR-L23), alveolar basal epithelial carcinoma (A549), amelanotic melanoma (C32) and melanoma (A375). The two reaction intermediates, **5a** and **5b,** showed the highest inhibition of NO production in murine monocytic macrophage (IC_{50} = 1.1 μM and IC_{50} = 2.3 μM respectively). Compound **5a** was the most active against melanotic melanoma (IC_{50} = 11.8 μM) while the other compounds exhibited weak cytotoxicity with IC_{50} values >50 μM on all cell lines.

Keywords: *Pyrano[3;2-e]indoles; indole derivatives; von Pechmann reaction; coumarin derivatives; inhibition of NO production; antiproliferative effect*

Introduction

Pyranoindoles constitute an important class of heterocyclic systems due to their remarkable biological activities. They are reported as μ-opiate receptor ligands [1], restricted phenolic analogs of the neurotransmitter serotonin [2]. They are inhibitors of hepatitis C polymerase [3], COX-2 [4], PLA2 and lipoxygenase [5]. They were reported also to possess anti-inflammatory [6] and antimicrobial activities [7]. Nevertheless, literature survey revealed that the pyrano[3,2-e] indole system has received little attention. The few reports on the derivatives of the latter system indicated that they act as CNS agents [8], inhibitors of poly-ADP-ribosylation reaction [9] and also restricted phenolic analogs of the neurotransmitter serotonin [2,10].

Several anti-inflammatory and neuroprotective drugs have been reported to have an antioxidant and/or radical scavenging mechanism as part of their activity [11–13]. The mechanism of inflammation injury is attributed, in part, to the release of reactive oxygen species (ROS) from activated neutrophiles and macrophages. The over production of these species lead to tissue injuries by damaging macromolecules and lipid peroxidation of the membranes [14–15]. In addition, ROS propagate inflammation by stimulating release of mediators such as NO and cytokines such as interleukin-1, tumor necrosis factor-α, and interferon-γ, which stimulate recruitment of additional neutrophiles and macrophages. Thus free radicals are important mediators that provoke inflammatory processes and, consequently, their neutralization by antioxidants and radical scavengers can attenuate inflammation [16–17].

Most clinically important medicines belong to steroidal or non-steroidal anti-inflammatory chemical therapeutics for treatment of inflammation-related diseases [18]. Although these drugs have a potent activity, yet a long-term administration is required for treatment of chronic diseases. Furthermore, these drugs have various severe side effects. Therefore, new naturally occurring agents with weak side-effects are desirable. Many coumarine- derivatives and analogs are able to scavenge reactive oxygen species (ROS)-free radicals, such as hydroxyl- and superoxide radicals,

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(Received 05 August 2008; revised 24 November 2008; accepted 07 December 2008)

ISSN 1475-6366 print/ISSN 1475-6374 online © 2009 Informa UK Ltd
DOI: 10.1080/14756360802693890
http://www.informahealthcare.com/enz

and also hypochlorous acid. Thus they can influence processes involving free radical-injury [19–20]. They have also been found to inhibit lipid peroxidation [21] and to possess vasorelaxant, anticoagulant, anti-inflammatory [22], antitumoral and anti-allergic [23] activities. Thus, on the basis of these evidences we wish to report herein a easy synthesis of pyrano[3,2-e]indoles **6a-b** and their precursors **4a-b** and **5a-b** using 5-hydroxyindole as a starting material.

Material & methods

Chemistry

Commercial reagents were purchased from Aldrich, Acros Organics and Alfa Aesar and used without additional purification. Melting points were determined on a Kofler melting point apparatus. IR spectra were taken with a Perkin Elmer BX FT-IR. Mass spectra were taken on a JEOL JMS GCMate spectrometer at ionising potential of 70 eV (EI) or were performed using a spectrometer LC-MS Waters alliance 2695 (ESI+). ^{1}H-NMR (400 MHz) was recorded on a JEOL Lambda 400 Spectrometer. Chemical shifts are expressed in parts per million downfield from tetramethylsilane as an internal standard. Thin layer chromatography (TLC) was performed on silica gel 60F-264 (Merck).

The von Pechmann reaction is a common method used to produce coumarins and derivatives [24]. Classically, the process consists of the condensation of phenols with β-ketoesters in the presence of an acidic catalyst (aluminium chloride, phosphorus pentoxide, trifluoroacetic acid [25]). Recently Indium(III) chloride [26] has been used as an efficient catalyst giving coumarins in good yields.

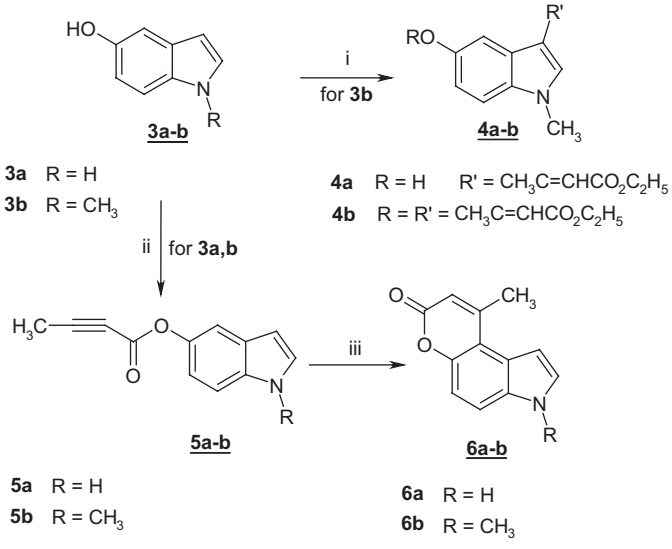
Accordingly, we attempted the von Pechmann reaction on 5-hydroxyindole or 5-hydroxy-1-methylindole using Indium(III) chloride as a catalyst for the preparation of compounds **6a-b** (Figure 1). Thus, when 5-hydroxy-1-methylindole **3b** was allowed to react with an equimolar amount of ethyl acetoacetate under von Pechmann conditions using Indium(III) chloride as a catalyst, the reaction product was found to be the derivative **4a** and not the expected pyranoindole **6b** (Scheme 1). When the reaction was repeated using an excess of ethyl acetoacetate, also no pyranoindole was formed and the product was identified as the indole derivative **4b**.

These failures prompted us to try an another synthetic strategy previously described in the literature [27] by esterification of the 5-hydroxyindoles **3a-b** with but-2-ynoic acid to give the esters **5a-b**. When these esters were heated under reflux in dioxane-dichloroethane in the presence of $PtCl_4$ as a catalyst, an intramolecular cyclization occurred giving 9-methyl-3*H*-pyrano[3,2-e]indol-7-one (**6a**), (40%) and 3,9-dimethyl-3*H*-pyrano[3,2-e]indol-7-one (**6b**) (45%) (Scheme 1).

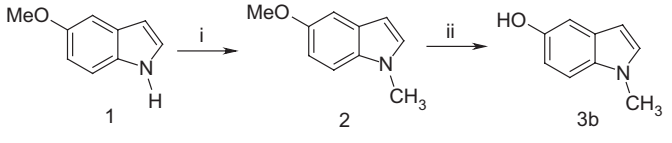
On the other hand, 5-hydroxy-1-methylindole **3b** could be prepared from 5-methoxyindole **1** by treatment with NaH to obtain the N-anion followed by reaction with methyl iodide to give 5-methoxy-1-methylindole **2**. Selective demethylation of the methoxyl group of compound **2** by heating under reflux in anhydrous pyridine hydrochloride gave the 5-hydroxy-1-methylindole **3b** (Scheme 2).

Procedure for the preparation of 5-methoxy-1-methylindole 2

To a stirred cold solution (0°C) of 5-methoxyindole (**1**) (2.0 g, 13.60 mmol) in dry DMF (60 mL), was added NaH 60% oil dispersion (0.54 g, 20.40 mmol). After 15 min stirring at this temperature, iodomethane (2.54 mL, 40.80 mmol) was added and the mixture was further stirred at room temperature for 1h. Water (200 mL) was then added to the reaction mixture and the solid product obtained was filtered, washed with water and dried. It is obtained as pure white solid, yield 92%, mp 114°C. IR (KBr): 2922, 1621, 1496, 1242, 1151, 1025, 802, 725 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 7.30 (d, 1H, Ar, J = 8.8 Hz); 7.23 (d, 1H, Ar, J = 2.9 Hz);); 7.04-7.01 (m, 1H, Ar); 6.76 (dd, 1H, Ar,



Scheme 1. Reagents: (i) ethyl acetoacetate (1 mol for **4a**, excess for **4b**); Indium(III)chloride; reflux 2h;(ii) but-2-ynoic acid; DCC; DMAP; CH_2Cl_2 / DMF (10:1); r.t. 2h; (iii) $PtCl_4$; 1,4-dioxane/1,2-dichloroethane (1:1); reflux 4h.



Scheme 2. Reagents: (i) NaH, CH_3I, DMF, 25°C; (ii) pyiridine hydrochloride; 3h reflux.

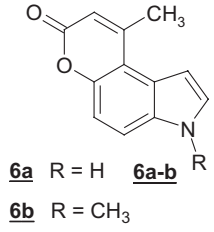


Figure 1. Structures of 6a and 6b.

J = 1.9, 8.8 Hz); 6.30 (d, 1H, Ar, J = 2.9 Hz); 3.72 (s, 6H, NCH_3 and OCH_3). MS (ESI^+): 162 (M^+ +1).

Procedure for the preparation of 5-hydroxy-1-methylindole 3b

A mixture of **2** and anhydrous pyridine hydrochloride (1:16) was heated to reflux for 3h. The reaction mixture was left to cool to room temperature, then ice water was added. The product was extracted with Et_2O. The organic layers were washed with a solution of HCl (2N), dried ($MgSO_4$) and concentrated to yield **3b** as yellow solid, yield 60%, mp = 156°C, IR (KBr): 3177, 2924, 1621, 1489, 1234, 1145, 949, 795, 719 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 8.67 (s, 1H, OH); 7.19 (s, 1H, Ar); 7.17-7.15 (m, 1H, Ar); 6.81 (d, 1H, Ar, J = 3.0 Hz); 6.62 (dd, 1H, Ar, J = 1.9, 8.8 Hz); 6.18 (d, 1H, Ar, J = 2.9 Hz); 3.04 (s, 3H, NCH_3). MS (ESI^+): 148 (M^+ +1).

General procedure for the preparation of Ethyl 3-(indol-3-yl)but-2-enoates 4a-b

To a mixture of 5-hydroxy-1-methyl-1*H*-indole (**3b**) and ethyl acetoacetate (equimolar ratio for **4a**, and excess for **4b**), indium(III) chloride (10 mol%) was added under nitrogen. The reaction mixture was heated under reflux for 2h, and then it was left to cool to room temperature. Ice water was added and then the reaction mixture was extracted by ethyl acetate. The organic layers were collected and washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The solid residue was washed with Et_2O, to give the pure compounds **4a-b**.

Ethyl 3-(5-hydroxy-1-methyl-1H-indol-3-yl)but-2-enoate **4a***:* pink solid, yield 58 %, mp = 200°C. IR (KBr): 3400, 2931, 1705, 1488, 1373, 1329, 1201, 1083, 1016, 851, 788 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 8.32 (s, 1H, Ar); 7.08-7.06 (m, 2H, Ar, OH); 6.52-6.49 (m, 2H, Ar); 6.45 (s, 1H, C=CH); 3.78-3.74 (q, 2H, CH_2); 3.67 (s, 3H, NCH_3); 1.83 (s, 3H, C-CH_3); 0.82-0.78 (t, 3H, CH_3). MS (EI) m/z: 259 (M^+, 1).

Ethyl 3-[5-(2-ethoxycarbonyl-1-methylvinyloxy)-1-methyl-1H-indol-3-yl]but-2-enoate **4b***:* pink solid, yield 65%, mp = 180°C; IR (KBr): 3412, 2984, 1705, 1622, 1473, 1373, 1168, 1088, 1027, 805 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 8.94 (s, 1H, Ar); 7.32 (d, 1H, Ar, $J_{7,6}$ = 8.8 Hz); 6.87 (s, 1H, Ar); 7.32 (d, 1H, Ar, $J_{6,7}$ = 8.8 Hz); 6.04-6.01 (m, 2H, C=CH); 4.11-4.09 (q, 2H, CH_2); 3.90-3.88 (q, 2H, CH_2); 3.76 (s, 3H, NCH_3); 1.42 (s, 6H, C-CH_3); 1.24-1.20 (t, 3H, CH_3); 0.95-0.92 (t, 3H, CH_3). MS (EI) m/z: 371 (M^+, 14).

General procedure for the preparation of indol-5-yl-but-2-ynoates 5a-b

To a solution of 5-hydroxyindole (**3a**) or 5-hydroxy-1-methyl-1*H*-indole (**3b**) in a mixture of CH_2Cl_2 and DMF (10:1), but-2-ynoic acid (1.1 eq), DCC (1 eq) and DMAP (cat) were added. The reaction mixture was stirred at room temperature for 2h, and then concentrated under reduced pressure. The solid residue was suspended in ethyl acetate, filtered and the filtrate was washed with brine. The collected organic layers were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The solid residue was purified by silica gel chromatography using ethyl acetate:petroleum ether, 2:8 as an eluent to give the pure compounds **5a** or **5b** respectively.

Indol-5-yl but-2-ynoate ***5a*****:** white solid, yield 62%, mp = 108°C. IR (KBr): 3410, 2237, 1705, 1480, 1261, 1121, 884 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 8.25 (bs, 1H, NH); 7.40 (s, 1H, Ar); 7.20-7.23 (m, 1H, Ar); 7.03-7.01 (m, 1H, Ar); 6.95 (dd, 1H, Ar, J = 1.9, 8.8 Hz); 6.60-6.50 (m, 1H, Ar); 2.10 (s, 3H, C-CH_3). GC-MS m/z: 199 [M^+].

1-Methyl-1H-indol-5-yl but-2-ynoate ***5b*****:** white solid, yield 64%, mp = 90°C; IR (KBr): 3402, 2233, 1711, 1625, 1487, 1246, 1217, 1123, 1033, 882 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 7.28 (s, 1H, Ar); 7.23-7.19 (t, 1H, Ar); 7.01 (d, 1H, Ar, J = 3.00 Hz); 6.98 (dd, 1H, Ar, J = 2.0, 8.8 Hz); 6.46 (d, 1H, Ar, J = 3.0 Hz); 3.70 (s, 3H, NCH_3); 2.06 (s, 3H, C-CH_3). MS (ESI^+): 214 (M^+ +1). GC-MS m/z: 213 [M^+].

General procedure for the preparation of pyrano[3,2-e]indoles 6a-b

To a solution of compound **5a** or **5b** (1 eq) in 1,4-dioxane and 1,2-dichloroethane (1:1) $PtCl_4$ (5 mol%) was added. The reaction mixture was heated under reflux for 4h then concentrated under reduced pressure. The residue was dissolved in ethyl acetate, and washed with brine. The organic layer was then dried ($MgSO_4$) and concentrated under reduced pressure to give a solid residue. The solid product was purified by silica gel chromatography using ethyl acetate:petroleum ether, (1:1) as an eluent to give the pure compounds **6a-b**.

9-Methyl-3H-pyrano[3,2-e]indol-7-one **6a***:* white solid, yield 45%, mp = 170°C. IR (KBr): 3400, 2925, 1700, 1660, 1610, 1580, 1410, 1248, 1050, 641 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 8.40 (bs, 1H, NH); 7.82 (d, 1H, Ar, J = 8.8 Hz); 7.70 (d, 1H, Ar, J = 3.0 Hz); 7.35 (d, 1H, Ar, J = 8.8 Hz); 6.94 (d, 1H, Ar, J = 3.0 Hz); 6.50 (s, 1H, Ar); 2.72 (s, 3H, CH_3). GC-MS m/z: 199 [M^+].

3,9-Dimethyl-3H-pyrano[3,2-e]indol-7-one **6b***:* white solid, yield 40%, mp = 154°C. IR (KBr): 3329, 2927, 1698, 1678, 1627, 1580, 1413, 1244, 1050, 641 cm^{-1}. ^{1}H-NMR (d_6-DMSO) δ 7.77 (d, 1H, Ar, J = 8.8 Hz); 7.58 (d, 1H, Ar, J = 2.9 Hz); 7.20 (d, 1H, Ar, J = 8.8 Hz); 6.86 (d, 1H, Ar, J = 2.9 Hz); 6.39 (s, 1H, Ar); 3.87 (s, 3H, NCH_3); 2.68 (s, 3H, CH_3). MS (ESI^+): 214 (M^+ +1).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

This experimental procedure was adapted from Wang et al. [28] modified as reported in Conforti et al. [29]. The absorbance was measured using a Perkin Elmer Lambda 40 UV/VIS spectrophotometer at 517 nm against blank, which was without DPPH. All tests were run in triplicate and averaged. Ascorbic acid was used as a positive control. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. This activity is given as % DPPH radical-scavenging that is calculated in the equation:

$$\%\,\text{DPPH radical} - \text{scavenging} = [1 - (\text{sample absorbance with DPPH} - \text{sample absorbance without DPPH/control absorbance})] \times 100.$$

Cell line and cell culture

The murine monocytic macrophage cell line RAW 264.7 (ECACC No.: 91062702) was grown in plastic culture flask in Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine supplemented with 10% foetal bovine serum (FBS) and 1% antibiotic/antimitotic solution (penicillin/streptomycin) under 5% CO_2 at 37°C. After 4-5 days cells were removed from culture flask by scraping and centrifugated for 10 min under 1500 rpm. The medium was then removed and the cells were resuspended with fresh DMEM. Four cancer cell lines, large lung cell carcinoma COR-L23 (ECACC No.: 92031919), amelanotic melanoma C32 (ATCC No.: CRL-1585), alveolar basal epithelial carcinoma A549 (EACC No.: 86012804) and melanoma were used in this experiment A375 (EACC No.: 88113005). The COR-L23, C32 cells were cultured in RPMI 1640 medium, while A549 and A375 cells were cultured in D-MEM medium. Both medium were supplemented with 10% foetal bovine serum, 1% L-glutamine, 1% penicillin/streptomycin.

Cells counts and viability were performed using a standard trypan blue cell counting technique. The cell concentration was adjusted to 1×10^6 cells/mL for RAW 264.7 and 2×10^4 for COR-L23, C32, A549 and A375 in the same medium. 100 µL of the above concentration were cultured in 96-well plate for one days to become nearly confluent. Concentrations ranging from 5–200 µg/mL of the samples were prepared from the stock solutions by serial dilution in DMEM to give a volume of 100 µL in each well of a microtiter plate (96-well). Then cells were cultured with vehicle, compounds for 48 h for human tumor cells and for 24 h in the presence of 1 µg/mL LPS for RAW 264.7.

Inhibition of nitric oxide (NO) production in LPS-stimulated RAW 264.7 cells

The presence of nitrite, a stable oxidized product of NO, was determined in cell culture media by Griess reagent (1% sulfanamide and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride in 2.5% H_3PO_4) [30]. 100 µL of cell culture supernatant was removed and combined with 100 µL of Griess reagent in a 96-well plate followed by spectrophotometric measurement at 550 nm using a microplate reader (GDV DV 990 B/V, Roma, Italy). Nitrite concentration in the supernatants was determined by comparison with a sodium nitrite standard curve.

Assay for cytotoxic activity

Cytotoxicity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) assay reported by Tubaro et al. [31] with some modification. All the cell lines used were capable of attachment to form a homogeneous monolayer on plastic substratum of the culture wells, which is ideal for the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

The assay for each concentration of samples was performed in triplicates and the culture plates were kept at 37°C with 5% (v/v) CO_2 for one days. After incubation, 100 µL of medium was removed from each well. Subsequently, 100 µL of 0.5% w/v MTT (Sigma, Italy), dissolved in phosphate buffered saline, was added to each well and allowed to incubate for a further 4 h. After 4 h of incubation, 100 µL of DMSO was added to each well to dissolve the formazan crystals. Absorbance values at 550 nm were measured with a microplate reader (GDV DV 990 B/V, Roma, Italy). Cytotoxicity was expressed as IC_{50} which is the concentration to reduce the absorbance of treated cells by 50% with reference to the control (untreated cells).

Statistical analysis

Data were expressed as means ± SD. Statistical analysis was performed by using Student's *t* test. Differences were considered significant at $P \leq 0.05$. The inhibitory concentration 50% (IC_{50}) was calculated from the Prism dose–response curve (Prism Graphpad, Prism version 4.0 for Windows, GraphPad Software, San Diego, CA, USA) obtained by plotting the percentage of inhibition versus the concentrations.

Results and discussion

All compounds prepared, **4a-b, 5a-b** and **6a-b**, were screened for their antioxidant activity, inhibition of NO production and also for their *in vitro* growth inhibitory activity of human tumor cell lines (C32: amelanotic melanoma; A375: melanoma; COR-L23: large lung carcinoma; A549: alveolar basal epithelial carcinoma). To evaluate the inhibition of NO production activity of the compounds prepared, the murine monocytic macrophage cell line RAW 264.7 was employed as a model for inhibition of inflammation mediators such as NO. Indomethacin was used as a reference drug. Once activated by inflammatory stimulation, macrophages produce a large number of cytotoxic molecules. It is well characterized that treatment of RAW 264.7 macrophages with LPS (1 µg/mL) for 24 h induces NO production (26.4 µM from the basal level of 0.6 µM) [32], as assessed by measuring the accumulation of nitrite, a stable metabolite of NO, in the media based on Griess reaction. The beneficial effect of coumarin derivatives on the inhibition of production of the inflammatory mediators in macrophages can be mediated through oxidative degradation of products of phagocytes, such as O^{2-} and HOCl. As shown in Table I, incubation of RAW 264.7 cells with prepared compounds induced a significant inhibitory effect on the LPS-induced nitrite production. At 50 µM compound **5a** is as efficient as **5b** with 99 (±2.2) and 97 (±2.1) % inhibition of LPS-induced NO production in RAW 264.7 cells (Figure 2) whereas the compound **5a** is more potent than **5b** with an IC_{50} value of 1.1 and 2.3 µM (Table I). These results are important if compared to the reference drug indomethacin which showed an IC_{50} of 147.5 µM. **4b** and **6a** showed activity similar to reference drug indomethacin with IC_{50} values of 38.9 and 43.7 µM (Table I and Figure 3). **4a** showed the lower activity with an IC_{50} value of 83.5 µM while **6b** didn't show any activity. All compounds did not show any cytotoxicity on RAW 264.7 cells at tested concentrations (data not shown).

Table I. IC_{50} values of inhibition of NO production in LPS-induced RAW 264.7 macrophages for compounds.

Compds	IC_{50}, μM[a]
4a	83.5 ± 2.3**
4b	38.9 ± 1.1**
5a	1.1 ± 0.1**
5b	2.3 ± 0.1**
6a	43.7 ± 1.1**
6b	>100
Indomethacin[b]	147.5 ± 1.2

[a]Values are means of three experiments, standard deviation is given in parentheses. [b]Indomethacin was used as positive control. ** $p < 0.01$.

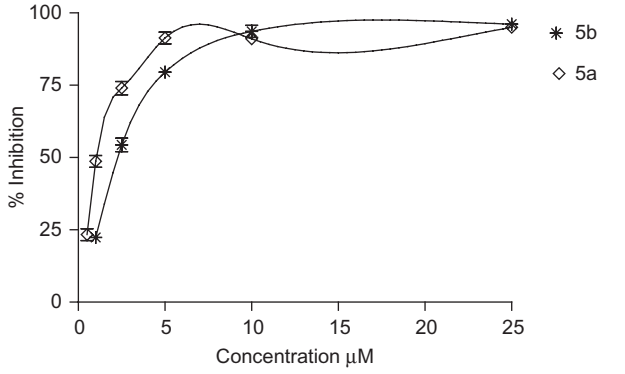


Figure 2. Percentage inhibition of NO production in LPS-induced RAW 264.7 macrophages for compounds **5a** and **5b**.

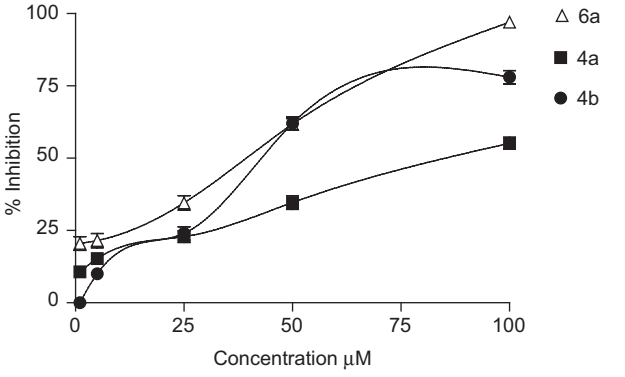


Figure 3. Percentage inhibition of NO production in LPS induced RAW 264.7 macrophages for compounds **6a, 4a** and **4b**.

Many non-steroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers [33]. Several compounds with antioxidant properties offer protection in rheumatoid arthritis and inflammation and find uses for the treatment of these pathologies [34]. Thus, we tested the new derivatives with regard to their antioxidant ability and in comparison with well-known antioxidant agents, for example, ascorbic acid. The interaction of the examined compounds with the stable free radical DPPH (1,1-diphenyl-2-picrylhydrazyl) [35] was studied at 1000 to 50 μM after 30 min (Table II). Compounds **4a-b** and **5a** interact with DPPH in a concentration dependent manner (Figure. 4), whereas compounds **6a-b** and **5b** do not show any interaction under tested concentrations. Slight differences are observed between the compounds **4b** and **5a** with the concentration whereas compound **4a** presents reducing ability at 100 μM.

Table II. Radical scavenging activity of analyzed compounds.

Comps	IC_{50},μM[a]
4a	75. 0 ± 0.9**
4b	712.1 ± 1.3**
5a	802.2 ± 3.1**
5b	>1000
6a	>1000
6b	>1000

[a]Values are means of three experiments, standard deviation is given in parentheses. ** $p < 0.01$. Ascorbic acid (2 μg/mL) was used as positive control.

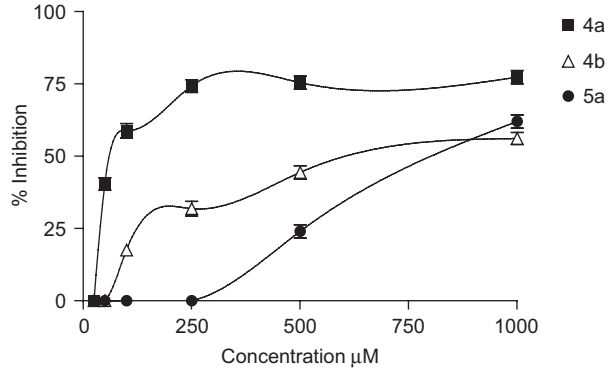


Figure 4. Percentage inhibition on DPPH radical of compounds **4a**, **4b** and **5a**.

Table III. Cytotoxic activities against human tumor cell lines of compounds.

	IC_{50} μM[a]			
Comps	COR-L23	A549	C32	A375
4a	>50	>50	>50	>50
4b	39.3 ± 1.3**	>50	>50	40.4 ± 1.4**
5a	38.9 ± 1.3**	>50	11.8 ± 0.5**	>50
5b	>50	>50	21.8 ± 0.9**	>50
6a	>50	>50	>50	>50
6b	>50	>50	>50	>50

[a]Values are means of three experiments, standard deviation is given in parentheses. ** $p < 0.01$. COR-L23: large lung carcinoma; A549: alveolar basal epithelial carcinoma; C32: amelanotic melanoma; A375: melanoma. Vinblastine (2 μg/mL) was used as positive control.

Furthermore the pyranoindoles and their synthetic precursors were evaluated for their *in vitro* cytotoxic properties on four human cancer cell lines. For each cell lines, there was a linear relationship between cell number and absorbance, measured at 550 nm in both control and drug-treated wells. After 48 hours of treatment, the cytotoxicity of the compounds under test was determined. The cytotoxic effects of indoles derivatives on the growth of human tumor cell lines are given in Table II. The **4a**, **6b** and **6a** compounds exhibited weak cytotoxicity with IC_{50} values >50 μM on all cell lines. The most antiproliferative compound against C32 cell line was **5a** followed by **5b** with IC_{50} values of 11.8 and 21.8 μM, respectively. **4b** showed the same activity on COR-

L23 and A375 cell lines with IC_{50} values of 39.3 and 40.4 μM, respectively (Table III).

Conclusion

In conclusion the antiradical activity of the tested compounds can explain, at least in part, the inhibition of NO production and antiproliferative activities. Compound **5a** is the most active *in vitro*. It was found to possess inhibitory activity against the production of NO and against the growth of human amelanotic melanoma cells (C32). Furthers investigations are in progress.

***Declaration of interest*:** The authors report no conflicts of interest.

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