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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

In-vitro cytotoxicity, antioxidant, bleomycin-dependent DNA damage and immunomodulatory evaluation of 1-(4-acetylphenyl)-3-aryloxypyrrolidine-2, 5-dione based derivatives

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Abstract A one pot, economical, and efficient synthesis of 1-(4-acetylphenyl)-3-aryloxypyrrolidine-2,5-diones-based derivatives 5a-l have been accomplished in single steps and in satisfactory yields from 1-(4-acetylphenyl)-pyrrole-2,5-diones 3 and phenols **4a–l**. All the compounds were characterized by physical, spectroscopic, and elemental analysis. The selection of the bioassays was based on proving the drug receptor binding concept. Compounds **5g**, **5k**, **5h**, **5i**, **5a**, and **5f** showed the highest inhibitory antioxidant activity using ABTS methods. Compounds **5k**, **5g**, **5c**, **5h**, **5b**, **5d**, **5f**, and **5j** manifested the best protective effect against DNA damage induced by bleomycin. Moreover, an in-vitro cytotoxic activity evaluation of all synthesized compounds was against four cancer cell lines using 5-Fluorouracil as a standard anticancer drug.

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Keywords Oxa-michael adducts · Antioxidant · Bleomycin-dependent DNA damage · Lymphocyte transformation · Cytotoxicity

Introduction

Every year almost 9 million cancer cases are newly diagnosed in developing countries where cancer incidence continues to increase at alarming rates. According to cancer society statistics, at least one third of these individuals are not expected to survive the disease, making cancer as the most prevalent cause of death. Systemic chemotherapy has emerged as a very promising strategy in treating a wide variety of cancers (Dhorajiya *et al*., 2014). Thus, the search for new ones has been prompted as urgency. Cyclic imides are an important class of molecules known for their diverse array of bioactivities (Kasimogullari and Cesur, 2004; Guzel *et al*., 2006). These biological activities and various pharmacological uses of cyclic imides have been attributed to their unique structural features, which encourage us to look for newer and more advantageous methods for their efficient synthesis. Reported methods of Michael addition on maleimides employed either the use of pyridine as base in ethanol or triethylamine as base in dichloromethane (Badawy *et al*., 1988; Pastor *et al*., 1985; Wabnitz *et al*., 2003). These methods suffer from disadvantages such as poor yield, longer reaction time, use of hazardous chemicals, and halogenated solvent. To nullify such predicaments, essential needs to develop reaction condition such that eco-friendly, highly efficient, better yielding, and take place in a short time.

In recent years, reaction in water has attained significant importance because of its advantages over other solvents. Water is one of the most environmentally benign and

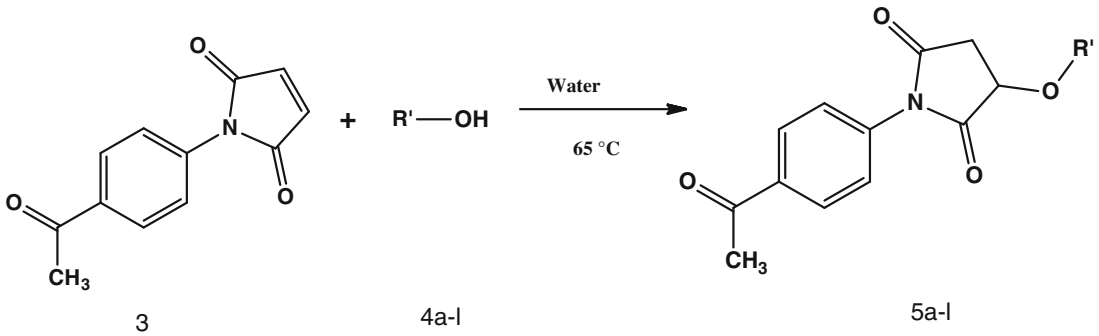
inexpensive liquid used in organic synthesis. Moreover, the reaction in water is greener approach to the synthesis of molecule of interest. Many organic reactions have been tried and tested in an aqueous medium with mixed results (Cheng and Comer, 2002; Zahouily *et al*., 2003; Beheshtia *et al*., 2010). The insolubility of most organic reagents in water has created a need to improve their compatibility in water with use of Phase-transfer catalyst, surfactants, or ionic liquids. Nevertheless, there are inherent difficulties in handling such reactions. In continuation of our work on the synthesis of biologically active motifs, we report here the preparation of a new series of Michael adducts with the objective of obtaining new biologically active compounds.

Result and discussion

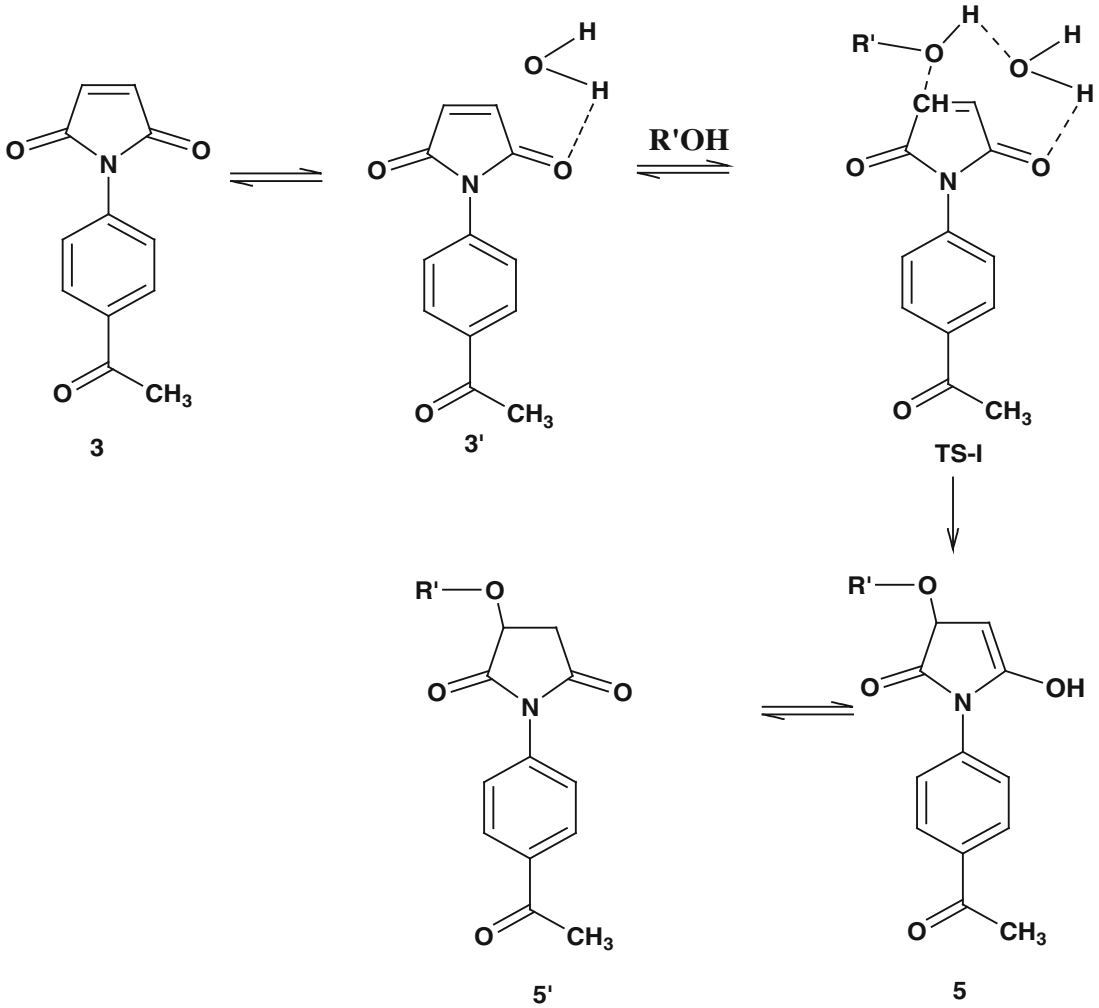
With an ever interesting quest for greener solvents and exploration of new reactions in water, we herein report the use of water for Michael addition of phenols to maleimide resulted afford products in good yield, within short reaction time, and in neutral conditions without use of any kind of catalyst. Maleimides were prepared by condensation of maleic anhydride with a different p-amino acetophenone using DABCO catalyst to give corresponding maleimide in excellent yield (Khatik *et al*., 2006). As a model reaction, 250 ml three necked flask equipped with a Teflon-coated 3.2-cm egg-shaped magnetic stirrer bar, internal temperature probe and a reflux condenser. Charged with distilled water, 1-(4-acetylphenyl)-pyrrole-2,5-diones (3) (0.21 g, 1 mmol, 1.00 equiv) and *p*-bromophenol (0.15 g, 1 mmol, 1.00 equiv) were added and the solution was stirred for 30 min. The reaction mixture was heated at 60–65 °C internal temperature for 1 h. After 1 h, TLC analysis revealed that the reaction was complete. The reaction flask was allowed to cool to ambient temperature. The reaction mixture was transformed to separating funnel and washed with saturated sodium bicarbonate solution and extracted with ethyl acetate. The combined organic layer was dried over $MgSO_4$ (6.00 g). The drying agent is removed by filtration before the organic layer was concentrated by rotary evaporation (The bath temperature is increased from 30 to 45 °C, 30–60 mmHg) yielding the crude product as brown solid. To generalize the reaction, conjugate addition with a different aromatic phenol were tried as well (Scheme 1). The reaction proceeded well in all cases, and the products were obtained within 1 h and 30 min with 70–93 % yields as shown in Table 1. Hydrogen-bond formation between water and the carbonyl oxygen of maleimide causes electrophilic activation, making the β-position more susceptible to neucleophilic attack. The oxygen atom of water in turn forms a hydrogen bond with the phenol hydrogen and increases the electron density at the oxygen, resulting in activation of nucleophile, which attacks the β-position of maleimide. This mechanism illustrated as in (Scheme 2) can be assumed to take place through the proposed transition state TS-I. Thus, because of hydrogen bonding between the carbonyl oxygen and water results in an increase the electrophilicity at β-carbon, and similarly the hydrogen bonding between the nucleophile and the water

Table 1 Isolated yield of 1-(4-acetylphenyl)-3-aryloxypyrrolidine-2,5-diones **5a–l**

Entry	R′	Isolated yield (%)
5a	α- Napthyl	85
5b	p-Br-C_6H_4	76
5c	m-Cresyl	70
5d	o-Cresyl	68
5e	p- Cresyl	72
5f	p-NO_2-C_6H_4	91
5g	o-COOH-C_6H_4	93
5h	o-CHO-C_6H_4	92
5i	2,4,6-[NO_2]-C_6H_4	94
5j	*p*-NH_2–C_6H_4	90
5k	Diphenyl	92
5l	*N*-CH_3-Quinolin-4-yl	90



Scheme 1 Synthesis of 1-(4-acetylphenyl)-3-aryloxypyrrolidine-2,5-diones **5a–l**



Scheme 2 Proposed mechanism for addition of phenols to maleimide

leads to nucleophilic activation. Here, the dual activation role of water can be seen (Blokzij *et al*., 1991).

Antioxidant screening assay (ABTS method)

2,2′-Azino-bis-3-ethylbenzthiazol-ine-6-sulfonic acid (ABTS) was purchased from Wako Co., USA. L-ascorbic acid was obtained from Sigma and all other chemicals were of the highest quality available. For each of the investigated compounds (2 ml), ABTS solution (60 µM) was added to 3 ml MnO_2 solution (25 mg/ml) all prepared in (5 ml) aqueous phosphate buffer solution (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered, and the absorbance of the resulting green–blue solution (ABTS radical solution) at 734 nm was adjusted to approx. 0.5. Then, 50 µl of (2 mM) solution of the tested compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured, and the reduction in color intensity was expressed as inhibition percentage. L-ascorbic acid was used as standard antioxidant (positive control) (Abdel-Wahab *et al*., 2009). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of tested compounds. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) only. The absorbance A(test) was measured and the reduction in color intensity was expressed as % inhibition. The inhibition for each compound was calculated from the following equation.

$$\% \text{ Inhibition} = [\text{A(control)} - \text{A(test)}/\text{A(control)}] \times 100$$

Ascorbic acid (vitamin C) was used as standard antioxidant (positive control). Blank sample was run without ABTS and using methanol/phosphate buffer (1:1) instead of sample. Negative control sample was run with methanol/phosphate buffer (1:1) instead of tested compound (Morimoto *et al*., 1995; Lissi *et al*., 1999).

A variety of N-(4-acetylphenyl) maleic imide-based Oxa Michael adducts were tested for antioxidant activity as reflected in the ability to control of ABTS assays. The pro-

oxidant activities of the aforementioned derivates were assessed by their effects on bleomycin-induced DNA damage. The *N*-(4-acetylphenyl) maleic imide-based Oxa Michael adducts manifested potent antioxidative activity in the ABTS assay. All compounds have been tested to bleomycin-dependant DNA damage. The results indicated that they may have some protective activity to DNA by certain mechanism. A series of compounds (**5g**, **5k**, **5h**, **5i**, **5a**, **5f**) exhibited a high antioxidant activity. On the other hand, compounds (**5k**, **5g**, **5c**, **5h**, **5b**, **5d**, **5f**, **5j**) protect the DNA from the induced damage by bleomycin (Table 2) (Abdel-Wahab *et al*., 2009; El-Gazzar *et al*., 2009).

Lymphocyte transformation assay

Lymphocyte transformation assay involves study of a specific immune response at 50 μM. The assay investigates the mitogenic effect of synthesized Michael adducts on T-Lymphocytes. In this assay, standard extract was observed 74 % at 50 μM. Data obtained from Table 2 indicated compound **5j** 67 % lymphocyte transformed at concentration similar to standard, while 50–55 % lymphocyte transformed by synthesized adducts **5k** and **5a** (Mikhaeil *et al*., 2004).

Table 2 Biological activities of compounds **5a–l**, toward lymphocyte transformation, ABTS (free radical scavenging), and bleomycin-dependent DNA damage activities

Comp. no.	Lymphocyte transformation assay at 50 μM[a,b,g]	ABTS (Percentage of scavenging inhibition)[c,d,e,f,g]	Bleomycin-dependent DNA damage assay[g,h]
5a	10	34.3 ± 0.10	0.210 ± 1.12
5b	50	37.5 ± 0.05	0.017 ± 0.15
5c	35	42.7 ± 1.15	0.015 ± 0.25
5d	35	36.8 ± 0.13	0.017 ± 0.04
5e	25	41.1 ± 2.38	0.080 ± 0.20
5f	20	35.4 ± 0.24	0.017 ± 0.05
5g	35	25.8 ± 1.30	0.014 ± 1.31
5h	45	33.2 ± 0.70	0.016 ± 0.27
5i	25	33.3 ± 0.14	0.110 ± 1.13
5j	67	69.5 ± 0.04	0.017 ± 0.03
5k	55	30.7 ± 1.16	0.011 ± 0.27
5l	39	46.8 ± 0.15	0.023 ± 0.03

[a] Concentration showing 50 % lymphocyte transformation

[b] The positive control was Echinacea purpurea (Ech) extract at concentration 50 gave 74 % lymphocyte transformation

[c] $ABTS^{+}$ Scavenging activity (%) = [Ac − As/Ac] × 100; where A_C is the absorbance value of the control and A_S is the absorbance value of the added samples test solution

[d] The concentration of the pure compounds was 2 mM

[e] The concentration showing 50 % inhibition is expressed in mM

[f] The positive control was vitamin C, and showed 80.0 ± 1.04 %

[g] Values are means of 3 replicates ± SD, and significant difference at $P < 0.05$ by Student's test

[h] The positive control was vitamin C, (0.24 mM)and showed absorbance 0.0038 ± 0.01 at the same concentration of the tested compounds

Cytotoxicity and antitumor assays

Cytotoxicity was expressed as the concentration that caused 50 % loss of the cell monolayer (IC_{50}). The assay was used to examine the newly synthesized compounds. 5-Fluorouracil as a standard anticancer drug was used for comparison. The results of our preliminary screening indicates that compound **5j** showed strong cytotoxic activity, where as compounds **5b** and **5l** showed moderate cytotoxic activity. The other compounds **5a** and **5c–h** showed weak cytotoxicity activity (Table 3).

The compounds **5i**, **5k**, and **5l** are strong to moderate activity against MCF-7 and HEPG-2 cell lines, while weak cytotoxic activity against WI-38 and VERO cell lines. Subsequently, we may conclude the following structure activity relationship's (SAR's). (1) The presence of basic skeleton (maleic imide moiety) is necessary for the broad spectrum of cytotoxic activity toward different cell lines (HepG2, WI-38, VERO, and MCF-7). (2) Introducing amino group (electron-donating group) in position 4 of phenol to form Michael adduct with maleic imide moiety strong activity toward all cell lines (compound **5j**). (3) Introducing one bromine atoms (electron-withdrawing group) in position 4 of phenol to form Michael adduct with

Table 3 In-vitro cytotoxic activities of compounds against four human cell lines

Compounds	IC_{50} (μM)[a]			
	WI-38	VERO	MCF-7	HEPG-2
5a	98 ± 0.08	84 ± 0.14	74 ± 1.34[b]	69 ± 0.29
5b	35 ± 0.17	28 ± 0.12	31 ± 1.32[b]	30 ± 0.29
5c	62 ± 0.05	59 ± 0.13	55 ± 1.30[b]	67 ± 0.15
5d	88 ± 0.12	84 ± 0.15	86 ± 1.74[b]	66 ± 0.23
5e	97 ± 0.13	90 ± 0.12	60 ± 1.32[b]	56 ± 0.21
5f	82 ± 0.12	70 ± 0.15	64 ± 1.25[b]	70 ± 0.23
5g	95 ± 0.19	88 ± 0.15	78 ± 1.35[b]	63 ± 0.21
5h	90 ± 0.14	89 ± 0.18	78 ± 1.24[b]	72 ± 0.21
5i	110 ± 0.05	95 ± 0.05	30 ± 0.05	25 ± 0.32
5j	15 ± 0.34	25 ± 0.11	20 ± 0.35	15 ± 0.35
5k	105 ± 0.12	85 ± 0.04	30 ± 0.01	25 ± 0.05
5l	50 ± 0.25	40 ± 0.22	35 ± 0.22	32 ± 3.01
5-FU	10 ± 0.05	08 ± 1.02	10 ± 0.48	05 ± 1.36

[a] (IC_{50}, μM): 1–10 (very strong), 11–25 (strong), 26–50 (moderate), 51–100 (weak), 100–200 (very weak), Above 200 (non-cytotoxic)

[b] Values are means of 3 replicates ± SD, and significant difference at $P < 0.05$ by Student's test

maleic imide moiety decreases the activity from strong to moderate against all cell lines (compound **5b**). (4) According to the above findings, the presence of one $-NO_2$ electron-withdrawing group in position 4 of phenol showed the weak cytotoxicity activity toward all cell lines (compound **5f**), while increases number of $-NO_2$ electron-withdrawing group in position 2, 4, 6 of phenol enhanced the cytotoxicity activity toward MCF-7 and HEPG-2 (compound **5i**). (5) In compound **5k**, the presence of phenyl group in position 4 of phenol to form Michael adduct with maleic imide moiety acts as electron-donating group showed nearly strong to moderate activity against HepG2, MCF-7, and weak activity against WI- 38, VERO. (6) Introducing heterocyclic phenol (*N*-methyl-4-hydroxy Quinoline) to form Michael adduct with maleic imide moiety decreases showed moderate cytotoxicity activity toward all cell lines (compounds **5l**). (7) In compounds **5c**–**d**, $-CH_3$ group at respective *o*, *m,* and *p*-position of phenol showed weak cytotoxicity activity toward all cell lines. Incorporation of weak electron-donating group irrespective of their position on phenol was not affect cytotoxically on all four cell lines (Fadda *et al*., 2012; Gutterdge *et al*., 1981).

Conclusion

We have demonstrated that the reaction of 1-(4-acetylphenyl)-pyrrole-2,5-diones with phenols via Michael addition reaction resulting 1-(4-acetylphenyl)-3-aryloxypyrrolidine-2,5-diones, enables the efficient synthesis of Michael adduct in single step in satisfactory overall yields. The products of this reaction are of potential medicinal interest. The present approach offers significant advantages over the previously described methods because of the ready availability of starting materials and simple operations. Moreover, we have shown from the biological evaluation part that most of the 1-(4-acetylphenyl)-3-aryloxypyrrolidine-2,5-dione derivatives have an excellent lymphocyte transformation, ABTS (free radical scavenging), bleomycine-dependent DNA damage properties, and in-vitro cytotoxic activities against four cell lines.

Experimental

Materials and instrumentation

All reagents were of analytical reagent grade and were used without further purification. Solvents employed were purified by standard procedure before using. Maleic anhydride, *p*-aminoacetophenone, and phenols were purchased from Aldrich.[12] Melting points were determined in open capillary on Veego (Model: VMP-D) electronic apparatus and are uncorrected. To monitor the reactions, as well as, to establish the identity and purity of reactants and products, thin layer chromatography was performed on microscopic glass slides (2×7.5 cm^2) coated with silica gel-G, using toluene–acetone and chloroform–methanol, as the solvent systems, and spots were visualized under UV radiation. Elemental analysis (C, H, N) was performed using a PerkinElmer, USA 2400-II CHN analyzer. FTIR spectra (4,000–400 cm^{-1}) were recorded on Simadzu 8400-S spectrophotometer using KBr disk. Nuclear magnetic resonance spectra were recorded on Varian 400 MHz model spectrometer using DMSO and or DMF as a solvent and TMS as internal reference (Chemical shifts in δ ppm).

Biological evaluation of the isolated compounds

DNA (Calf Thymus type1), bleomycin sulfate, butylated hydroxyanisole, thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), and ascorbic acid were obtained from sigma. 2,2′-azo-bis-(2-amidinopropane) dihydrochloride and ABTS were purchased from Wako Co., USA.

Bleomycin-dependent DNA damage assay

The reaction mixture contained DNA (0.5 mg/ml), bleomycin sulfate (0.05 mg/ml), $MgCl_2$ (5 mM), $FeCl_3$ (50 mM), and samples to be tested in a conc. of 0.1 mg/ml. L-ascorbic acid was used as positive control. The mixture was incubated at 370 °C for 1 h. The reaction was terminated by addition of 0.05 ml EDTA (0.1 M). The color was developed by adding 0.5 ml TBA (1 % w/v) and 0.5 ml HCl (25 % v/v), followed by heating at 80 °C for 10 min. After centrifugation, the extent of DNA damage was measured by increase in absorbance at 532 nm (Abdel-Wahab *et al*., 2009; Gutterdge *et al*., 1981).

Antioxidant activity screening assay 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid method

For each of the investigated compounds, 2 ml of ABTS solution (60 μM) was added to 3 ml MnO_2 solution (25 mg/ml), all prepared in 5 ml aqueous phosphate buffer solution (pH, 7; 0.1 M). The mixture was shaken, centrifuged, filtered, and the absorbance of the resulting green–blue solution (ABTS radical solution) at λ 734 nm was adjusted to approximately ca. 0.5. Then, 50 μl of (2 mM) solution of the tested compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in color intensity was expressed as inhibition percentage. L-ascorbic acid was used as standard antioxidant (positive control). Blank sample was run without ABTS and using MeOH/phosphate

buffer (1:1) instead of tested compounds (El-Gazzar *et al*., 2009).

Lymphocyte transformation assay

The viable lymphocytes were adjusted to a concentration of 2×10^6 cells/ml in RPMI-1640 medium supplemented with 600 µl penicillin, 0.1 ml streptomycin, 1 % glutamine, 25 % HEPES (*N*-2-hydroxyethylpiperazine-*N*-D2-ethanesulfonic acid)-buffer, and 20 % fetal calf serum (FCS). The lymphocytes were plated into 96-well tissue culture plates (or Ependorf tubes). 100 µl of the volatile oil solution in DMF (100 µl/ml) and 20 µg of the mitogen (PHA) were added to each well. Cell cultures were incubated at 37 °C in 5 % CO_2 atmosphere for 72 h, during which the mitogen produces its maximal effect on DNA synthesis. After culture, B. R. Mikhaeil et al. Chemistry and Immunomodulatory Activity of Frankincense Oil 237 cell films were stained by Giemsa stain and average count of percentage of transformed (proliferated) blasts was determined. Aqueous Echinaceae purpurea extract (Immulone) and levamisole (Ketrax) were used as positive control (standard immunostimulant) 100 µg/ml of each drug in DMSO.

Cytotoxicity and antitumor assay

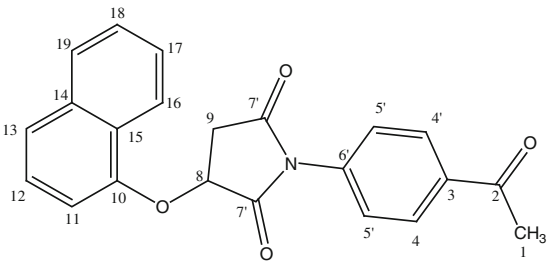
Samples were prepared for assay by dissolving in 50 ml of DMSO (Dimethyl Sulfoxide), and diluting aliquots into sterile culture medium at 0.4 mg/ml. These solutions were sub-diluted to 0.02 mg/ml in sterile medium and the two solutions were used as stocks to test samples at 100, 50, 20, 10, 5, 2, and 1 mg/ml in triplicate in the wells of microtiter plates. The compounds were assayed in triplicate on monolayers grown in Dulbecco's modified Eagle's medium supplemented with 10 % (v/v) calf serum (HyClone Laboratories, Ogden, UT), 60 mg/ml Penicillin G, and 100 mg/ml streptomycin sulfate maintained at 37 °C in a humidified atmosphere containing about 15 % (v/v) CO_2 in air. All medium components were obtained from Sigma Chemical Co., St. Louis, MO, unless otherwise indicated. Cell stocks were maintained at 34 °C in culture flasks filled with medium supplemented with 1 % (v/v) calf serum. Subcultures of cells for screening were grown in the wells of microtiter trays (Falcon Microtest III 96-wells trays, Becton–Dickinson Labware, Lincolin Park, NJ) by suspending cells in medium following trypsin–EDTA treatment, counting the suspension with a hemocytometer, diluting in medium containing 10 % calf serum to 2×10^4 cells per 200 ml culture, aliquoting into each well of a tray, and culturing until confluent. Microtiter trays with confluent monolayer cultures of cells were inverted, the medium shaken out, and replaced with serial dilutions of sterile compounds in triplicate in 100 l medium followed by titered virus in 100 ml medium containing 10 % (v/v) calf serum in each well. In each tray, the last row of wells was reserved for controls that were not treated with compounds. Trays were cultured for 96 h. Trays were inverted onto a paper towel pad, the remaining cells were rinsed carefully with medium, and fixed with 3.7 % (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water, and examined visually. The cytotoxic activity is identified as confluent, relatively unaltered monolayers of stained cells treated with the investigated compounds. Cytotoxicity was estimated as the concentration that caused approximately 50 % loss of the monolayer. 5-fluorouracil was used as a positive control.

Synthesis of 4′-amino maleimide 3

Mixer of maleic anhydride (1) and *p*-amino acetophenone (2) (1:1.1) in diethyl ether, catalyzed by DABCO (1,4-Diazabicyclo [2.2.2] octane) (0.5 mol) in short reaction time and high yield was performed to give 4′-amino maleimide (3) (Scheme 1) (Khatik *et al*., 2006).

General Procedure for the Preparation of 1-(4-acetylphenyl)-3 aryloxypyrrolidine-2,5-dione (**5a–l**)

In a typical experiment, 1-(4-acetylphenyl)-pyrrole-2,5-diones (3) (0.21 g, 1 mmol, 1.00 equiv) was taken in a round-bottomed flask containing mixture (1:1) of demineralized water, and 4-bromophenol (4d) (0.15 g, 1 mmol) was added. The Reaction vessel was subjected to heat for 1 h at temperature 60–65 °C, after that the reaction mixture was washed with saturated sodium bicarbonate solution and extracted with ethyl acetate. The solvent was evaporated under reduced pressure to obtain the product 1-(4-acetylphenyl)-3-(4-bromophenoxy) pyrrolidine-2,5-diones, which was washed with hexane and dried under vacuum.



*1-(4-Acetylphenyl)-3-(1-napthyloxy)-pyrrolidine-2,5-dione (**5a**)*

Brown solid. Yield 85 %; M.p. 145 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.55 (s, 3H); 3.11

(s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.64–8.17 (m, 7H), 7.32 (dd, J = 15, 1H), 7.34 (dd, J = 15, 2H).^{13}C NMR (500 MHz, DMSO-d_6); 22 (C-1), 32 (C-9), 80.8 (C-8), 103 (C-5, C-5′), 120 (C-12, C-16), 120.1 (C-17), 125 (C-4, C-4′), 126 (C-11, C-14, C-18, C-19), 127 (C-3), 129 (C-13), 133 (C-6), 145 (C-10), 170.9(C-7, C-7′), 191 (C-2) δ ppm; ESIMS *m/z* 359(M^+) Anal. Calc. for $C_{22}H_{17}NO_4$ (359.37): C, 73.53; H, 4.77; N, 3.90 Found: C, 73.51; H, 4.75; N, 3.88.

*1-(4-Acetylphenyl)-3-(4-Bromophenyloxy)-pyrrolidine-2,5-dione (**5b**)*

Brown solid. Yield 76 %; M.p. 166 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 7.32 (dd, J = 10, 1H), 7.34 (dd, J = 10, 2H).^{13}C NMR (500 MHz, DMSO-d_6); 22.8 (C-1), 32.2 (C-9), 83.2 (C-8), 115.4 (C-13), (C-5, C-5′), 116.3 (C-11, C-15), 120.3 (C-17), 128 (C-4, C-4′),132.4 (C-3), 133 (C12, C-14), 145 (C-6), 159 (C-10), 161(C-7, C-7′), 195 (C-2) δ ppm; ESIMS *m/z* 387 (M^{-1}); Anal. Calc. for $C_{18}H_{14}BrNO_4$ (388.21): C, 55.69; H, 3.63; N, 3.61 Found: C, 55.63; H, 3.62; N, 3.63.

*1-(4-Acetylphenyl)-3-(3-methylphenyloxy)-pyrrolidine-2,5-dione (**5c**)*

Brown solid. Yield 70 %; M.p. 149 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 7.32 (dd, J = 10, 1H), 7.34 (dd, J = 10, 2H).^{13}C NMR (500 MHz, DMSO-d_6); 11 (C- of m-Ar-CH_3), 22 (C-1), 31 (C-9), 80 (C-8),114 (C-15), 120 (C-11), 126.9 (C-13), 127.85 (C-5, C-5′), 129 (C-4, C-4′), 130.22 (C-14), 133 (C-3), 137 (C-12), 138 (C-6), 163 (C-10), 167.78 (C-7,C-7′), 171 (C-2) δ ppm; ESIMS *m/z* 324 (M^+) Anal. Calc. for $C_{19}H_{17}NO_4$ (323.34): C, 70.58; H, 5.38; N, 4.33 Found: C, 70.58; H, 5. 36; N, 4.32.

*1-(4-Acetylphenyl)-3-(2-methylphenyloxy)-pyrrolidine-2,5-dione (**5d**)*

Brown solid. Yield 68 %; M.p. 105 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 7.32 (dd, J = 10, 1H), 7.34 (dd, J = 10, 2H). ^{13}C NMR (500 MHz, DMSO-d_6); 11 (C- of o-Ar-CH_3), 22.3 (C-1), 31 (C-9), 80.7 (C-8),114 (C-15), 120 (C-13), 126.9 (C-5, C-5′), 127.85 (C14), 128 (C-11), 129 (C-4, C-4′), 130.22 (C-12), 133 (C-3), 138 (C-6), 163 (C-10), 167.78 (C-7, C-7′), 171 (C-2) δ ppm; ESIMS *m/z* 324 (M^+) Anal. Calc. for $C_{19}H_{17}NO_4$ (323.34): C, 70.58; H, 5.38; N, 4.33 Found: C, 70.56; H, 5. 34; N, 4.31.

*1-(4-Acetylphenyl)-3-(4-methylphenyloxy)-pyrrolidine-2,5-dione (**5e**)*

Orange brown solid. Yield 72 %; M.p. 152 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 7.32 (dd, J = 10, 1H), 7.34 (dd, J = 10, 2H). ^{13}C NMR (500 MHz, DMSO-d_6); 11.2 (C-of p-Ar-CH_3), 23 (C-1), 31 (C-9), 83 (C-8), 114 (C-11, C-15), 120 (C-5, C-5′), 130.22 (C-12, C-14), 133 (C-13), 138 (C-6), 163 (C-10), 167.78 (C-7, C-7′), 171 (C-2) δ ppm; ESIMS *m/z* 324 (M^+) Anal. Calc. for $C_{19}H_{17}NO_4$ (323.34): C, 70.58; H, 5.38; N, 4.33 Found: C, 70.58; H, 5. 33; N, 4.33.

*1-(4-Acetylphenyl)-3-(4-nitrophenyloxy)-pyrrolidine-2,5-dione (**5f**)*

Brownish black solid. Yield 91 %; M.p. 98 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 8.32 (dd, J = 15, 1H), 8.34 (dd, J = 15, 2H).^{13}C NMR (500 MHz, DMSO-d_6); 22.8 (C-1), 31 (C-9), 81.7 (C-8), 114 (C-11, C-15), 120 (C5, C-5′), 126.9 (C-12, C-14), 129 (C-4, C-4′), 133 (C-6), 135.9 (C-3), 138 (C-13), 163 (C-10), 167.78 (C-7, C-7′), 171 (C-2) δ ppm; ESIMS *m/z* 354 (M^+) Anal. Calc. for $C_{18}H_{14}N_2O_6$ (354.31): C, 61.02; H, 3.98; N, 7.91 Found: C, 59.99; H, 4.01; N, 7.89.

*2-((1-(4-Acetylphenyl)-2,5-dioxopyrrolidin-3-yl)oxy)benzoic acid (**5g**)*

Light brown solid. Yield 93 %; M.p. 115 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 7.34 (m, 4H), 10.2 (s, 1H).^{13}C NMR (500 MHz, DMSO-d_6); 22.8 (C-1), 31 (C-9), 80.7 (C-8), 114 (C-15), 120 (C-13), 126.9 (C-5, C-5′), 127.85 (C-4, C-4′), 130.9 (C-12), 129 (C-6), 133 (C-14), 135.9 (C-6), 138 (C-3), 163 (C-10), 167.78 (C of o-Ar-COOH), 171 (C-7, C-7′), 189 (C-2) δ ppm; ESIMS *m/z* 355 (M^{+2}) Anal. Calc. for $C_{19}H_{15}NO_6$ (353.32): C, 64.59; H, 4. 28; N, 3.96 Found: C, 64.57; H, 4.29; N, 4.0.

*2-((1-(4-Acetylphenyl)-2,5-dioxopyrrolidin-3-yl)oxy)benzaldehyde (**5h**)*

Light orange solid. Yield 92 %; M.p. 128 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 7.32 (m, 4H), 7.34 (dd, J = 10, 2H), 8.7 (s, 1H).13C NMR (500 MHz, DMSO-d_6); 22.8 (C-1), 31 (C-9), 80.7 (C-8), 114 (C-15), 120 (C-13), 121 (C-5, C-5′), 126.9 (C-11), 127.85 (C-12), 129.0 (C-4, C-4′), 135.9 (C-14), 137 (C-6), 138 (C-3), 163 (C-10), 168 (C-7, C-7′), 174 (C-2) δ ppm; ESIMS *m/z* 337 (M^+) Anal. Calc. for $C_{19}H_{15}NO_5$ (337.32): C, 67.65; H, 4. 48; N, 4.15 Found: C, 67.63; H, 4.46; N, 4.11.

*1-(4-Acetylphenyl)-3-(2,4,6-trinitrophenoxy)pyrrolidine-2,5-dione (**5i**)*

Yellow solid. Yield 94 %; M.p. 98 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 8.32 (dd, J = 15, 1H), 8.34 (dd, J = 15, 2H).^{13}C NMR (500 MHz, DMSO-d_6); 22.8 (C-1), 31 (C-9), 81.7 (C-8), 120 (C-5, C-5′), 126.9 (C-12, C-14), 127.85 (C-4, C-4′), 133 (C-6), 135.9 (C-7), 137 (C-13), 138 (C-11, C-15), 163 (C-10), 167.78 (C-7, C-7′), 171 (C-2) δ ppm; ESIMS *m/z* 354 (M^+) Anal. Calc. for $C_{18}H_{14}N_2O_6$ (354.31): C, 61.02; H, 3.98; N, 7.91 Found: C, 59.99; H, 4.01; N, 7.89.

*1-(4-Acetylphenyl)-3-(4-aminophenyloxy)-pyrrolidine-2,5-dione (**5j**)*

Dark brown solid. Yield 90 %; M.p. 98 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 8.32 (dd, J = 15, 1H), 8.34 (dd, J = 15, 2H).^{13}C NMR (500 MHz, DMSO-d_6); 22.8 (C-1), 31 (C-9), 81.7 (C-8), 114 (C-11, C-15), 120 (C-12, C-14), 126.9 (C-5, C-5′), 127.85 (C-4, C-4′), 133 (C-6), 135.9 (C-3), 138 (C-13), 163 (C-10), 167.78 (C-7, C-7′), 171 (C-2) δ ppm; ESIMS *m/z* 354 (M^+) Anal. Calc. for $C_{18}H_{14}N_2O_6$ (354.31): C, 61.02; H, 3.98; N, 7.91 Found: C, 59.99; H, 4.01; N, 7.89.

*1-(4-Acetylphenyl)-3-(biphenyloxy)-pyrrolidine-2,5-dione (**5k**)*

White solid. Yield 92 %; M.p. 98 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 8.32 (dd, J = 15, 1H), 8.34 (dd, J = 15, 2H).^{13}C NMR (500 MHz, DMSO-d_6); 22.8 (C-1), 31 (C-9), 81.7 (C-8), 114 (C-11), 120 (C-13), 126.9 (C-11, C-11′), 127.85 (C-13′), 128 (C-12), 129 (C–C-4, C-4′, C-12′), 130.22 (C-14), 133 (C-15), 135.9 (C-6), 137 (C-3), 138 (C-10′), 163 (C-10), 167.78 (C-7, C-7′), 171 (C-2) δ ppm; ESIMS *m/z* 354 (M^+) Anal. Calc. for $C_{18}H_{14}N_2O_6$ (354.31): C, 61.02; H, 3.98; N, 7.91 Found: C, 59.99; H, 4.01; N, 7.89.

*1-(4-Acetylphenyl)-3-(N-methyl-4-quinolinyloxy)-pyrrolidine-2,5-dione (**5l**)*

Dark orange solid. Yield 90 %; M.p. 98 °C (hexane/MeOH). FTIR (KBr): 1724, 1599, 1520, 1344, ^{1}H NMR (500 MHz, DMSO-d_6); 3.45 (DMSO solvent); 2.04 (s, 3H); 2.5 (s, J = 5, 1H); 5.3 (s, J = 10, 1H), 6.52 (dd, J = 10, 1H), 6.55 (dd, J = 10, 1H), 8.32 (dd, J = 15, 1H), 8.34 (dd, J = 15, 2H).^{13}C NMR (500 MHz, DMSO-d_6); 22.8 (C-1), 31 (C-9), 45.3 (C –of N-CH_3), 81.7 (C-8), 104.9 (C-11), 108.8 (C-18), 114 (C-16), 120 (C-14), 126.9 (C-5, C-5′), 127.85 (C-15), 128 (C-17), 129 (C-4, C-4′), 130.22 (C-12), 133 (C-6), 135.9 (C-3), 138 (C-13), 167.78 (C-7, C-7′), 171 (C-2) δ ppm; ESIMS *m/z* 354 (M+) Anal. Calc. for $C_{18}H_{14}N_2O_6$ (354.31): C, 61.02; H, 3.98; N, 7.91 Found: C, 59.99; H, 4.01; N, 7.89.

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