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Characterization, Cytotoxicity and Antimicrobial Studies of *(Z)*-2-(3-(butylamino)-4-oxopent-2-en-2-yl)-2-hydroxy-1H-indene 1,3(2H)-dione

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ABSTRACT

Most of the medicines which are currently used were discovered by empirical synthesis. The chance of obtaining newer medicines increases through simulation studies in biological and chemical properties of the newly synthesized compounds. In this study we reported a convenient synthesis of (Z)-2-hydroxy-2-(4-oxo-3-(propylamino)pent-2-en-2-yl)-1H-indene-1,3(2H)-dione and evaluated this synthesized compound for cytotoxic and antimicrobial activities.

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INTRODUCTION

During the last few decades, a central objective in synthetic organic chemistry has been to develop efficient syntheses of biologically active compounds with potential application in the pharmaceutical or agrochemical industries[1-8]. The growing incidence of microbial resistance to currently used antibiotics represents a serious medical problem. Therefore, there is an urgent need to develop new classes of therapeutic agents to treat microbial infections. Besides the exploitation of new targets, there is another approach of merging two or more pharmacophores into a single molecule. This approach can also reduce unwanted side effects. The success of this hybridization approach has already been applied in developing novel antibacterial and antimalarial agents to overcome drug resistance[9]. Cancer has become the second cause of mortality in the world and the development of potent and specific anticancer agents is urgently needed because of the problems like severe toxicity as well as resistance to the existing drugs[10]. The discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology for the control of infectious diseases. However, the increasing antimicrobial resistance emergence and its dissemination among bacterial strains reduced the efficiency of treatment success of large amount of drugs. Millions of organic chemical compounds are synthesized, hundreds of thousands of which have been tested to find new prospective leads for different pharmaco therapeutic areas. As a part of our current studies on the development of new routes towards pharmaceutical organic molecules we describe an efficient synthesis of *(Z)*-2-(3-(butylamino)-4-oxopent-2-en-2-yl)-2-hydroxy-1H-indene-1,3(2H)-dione through the reaction of *(E)*-4 (butylamino)pent-3-en-2-one with 1*H*-indene-1,2,3-trione (Ninhydrine) in MEK at room temperature. This new cascade synthetic method seems facile; workup procedure is easy and gives pure target compound in excellent yield and containing several potential centers for further modification. The synthesized compound was also evaluated for antimicrobial and cytotoxic activities.

MATERIAL AND METHODS

1.1. Chemistry:

Melting points were determined on a Kofler apparatus and are uncorrected. Enaminone was prepared a by known method[11] and other Chemicals were purchased from Fluka and were used without further purification; IR

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spectra: Shimadzu IR-460 spectrometer; ^{1}H- and ^{13}C-NMR spectra: Bruker DRX-75AVANC instrument; in $CDCl_3$ at 75 and 75 MHz, respectively, δ in ppm, J in Hz; EI-MS (70 eV): Elemental analyses (C, H, and N) were performed with a Heraeus CHN-O-Rapid analyzer. The results agreed favorably with the calculated values.

1.2. General procedure for the synthesis of the synthesized compound 3:

A solution of *(E)*-4-(butylamino)pent-3-en-2-one **1** (2 mmol) and 1*H*-indene-1,2,3-trione (Ninhydrine) **2** (2 mmol) in 5 ml of MEK at room temperature were magnetically stirred over 15 minutes. The precipitate was filtered and recrystallized from methanol to give *(Z)*-2-(3-(butylamino)-4-oxopent-2-en-2-yl)-2-hydroxy-1H-indene 1,3(2H)-dione **3** (Scheme 1).

(Z)-2-(3-(butylamino)-4-oxopent-2-en-2-yl)-2-hydroxy-1H-indene-1,3(2H)-dione (3):

Pale yellow powder, mp: 215–217 °C; yield: 0.30 g (96%). IR (ν_{max}/cm^{-1}): 3515 (NH), 3473 (OH), 1755, 1715, 1705 (3C=O). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00 (3H, t, $^{3}J_{HH}$= 7.2 Hz, Me), 1.47 (2H, m, C*H*$_2$), 1.55 (2H, m, C*H*$_2$), 1.75 (1H, broad s, OH), 2.36 (3H, s, Me), 2.45 (3H, s, Me), 3.74 (2H, m, C*H*$_2$ linked to N), 3.81 (1H, m, NH), 7.56-7.89 (4H, m, 4CH-Aro). ^{13}C NMR (75 MHz, $CDCl_3$): δ 13.1 (*C*H$_3$), 14.5 (*C*H$_3$), 20.9 (*C*H$_2$), 33.3 (*C*H$_3$), 42.8 (*C*H$_2$), 58.9 (*C*H$_2$N), 99.2 (C), 109.1 (C), 124.6 (2*C*H), 137.3 (*2*CH), 141.5 (2C), 148.7 (=C llinked to N), 194.1 (2C=O), 201.1 (C=O). EI-MS: *m/z* (%)= 315 (M+, 15), 272 (11), 258 (3), 231 (5), 216 (28), 171 (5), 104 (100), 84 (38), 76 (82), 57 (12). Anal. Calcd for $C_{18}H_{21}NO_4$ (315): C, 68.55%; H, 6.71%; N, 4.44%; found: C, 68.50%; H, 6.66%; N, 4.39%.

1.3. Biological Activity:

1.4. Assessment of cytotoxic activiy by Cell viability assay (MTT):

The cytotoxic activity of the synthesized compound was assessed at various concentrations against Glioma brain tumor animal model (C6) using the 3-(4,5-dimethylthiazol-2-yl)-2,5diphenyl tetrazolium bromide (MTT) assay[12]. Briefly 100 µl of culture medium containing 10000 C6 (Glioma Cell line) cells was decanted in 96-well plates and incubated (5% CO_2 and 37°C). The supernatant of cells was removed after 24 hours and different concentrations of (Z)-2-(3-(butylamino)-4-oxopent-2-en-2-yl)-2-hydroxy-1H-indene 1,3(2H)-dione was poured on cells and then incubated for 24 hours. Afterward, the supernatant was removed and 100 µl of MTT solution (0.5 mg/ml) was added. Three hours after incubation, purple color in live cells (due to the formation of Formazane) was dissolved in 100 µl of Isopropanol and absorbance was measured at 570 nm by the Power Eave XS spectrophotometer. Thereupon IC_{50} was calculated using Pharm program. Thus drug toxicity was investigated on Glioma cell line (C6) in different concentrations based on MTT assay and IC_{50} was obtained about 282.3 ± 4.6 µg/ml using Pharm program.

1.5. Assessment of antimicrobial activity by disc diffusion assay:

The disc diffusion method was one of two methods used for assessment of antimicrobial activity of the synthesized compound. The bacterial growth inhibitory test was carried out by the disc diffusion method according to the procedure of Murray *et al*[13]. The dried synthesized compound was dissolved in DMSO to a final concentration of 30 mg/ml and filtered by 0.45 ml Millipore filters for sterilization, Using 100 µl of suspension containing 108 CFU/ml of bacteria and 104 spore/ ml of fungi spread on the nutrient agar (NA) and potato dextrose (PD) agar mediums, respectively. Disk diffusion susceptibility studies were performed with Mueller-Hinton agar plates of 6-mm thickness and commercial antibiotic discs. The discs (6 mm in diameter) impregnated with 10 µl of the aforementioned solution (300 µg/ disc) and DMSO (as negative control) were placed on the inoculated agar. Zone diameters of inhibition measured after 24 h of incubation at 35°C and again after an additional 24 h of incubation at 22°C and each assay were repeated twice. Ampicillin (5 µg/disc) and gentamicin (10 µg/disc) and were used as positive controls for bacteria and ketoconazole (100 IU) for fungi.

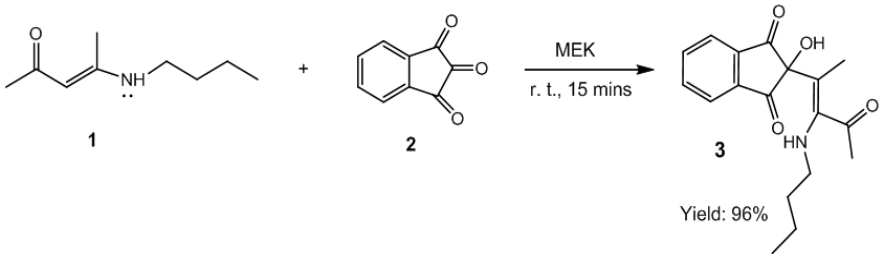
1.6. MIC agar dilution assay:

The minimum inhibitory concentrations (MICs) were measured as described previously[14] Initial emulsion of the synthesized compound **3** was prepared by addition to sterile molted SDA medium containing Tween 20 (0.5%, v/v). The resulting SDA agar solutions were immediately mixed and poured into microtiter plate (96 wells). The plate was spot inoculated with 108 CFU/ml (5µl) of bacteria and (5µl) 104 spore/ ml of fungus isolate. At the end of incubation period, the plates were evaluated for the presence or absence of growth. Ampicillin, Tetracycline and Fluconazole were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively. MICs were determined as the lowest concentrations preventing visible growth. Each test was repeated at least twice.

RESULTS AND DISCUSSION

1.7. Chemistry:

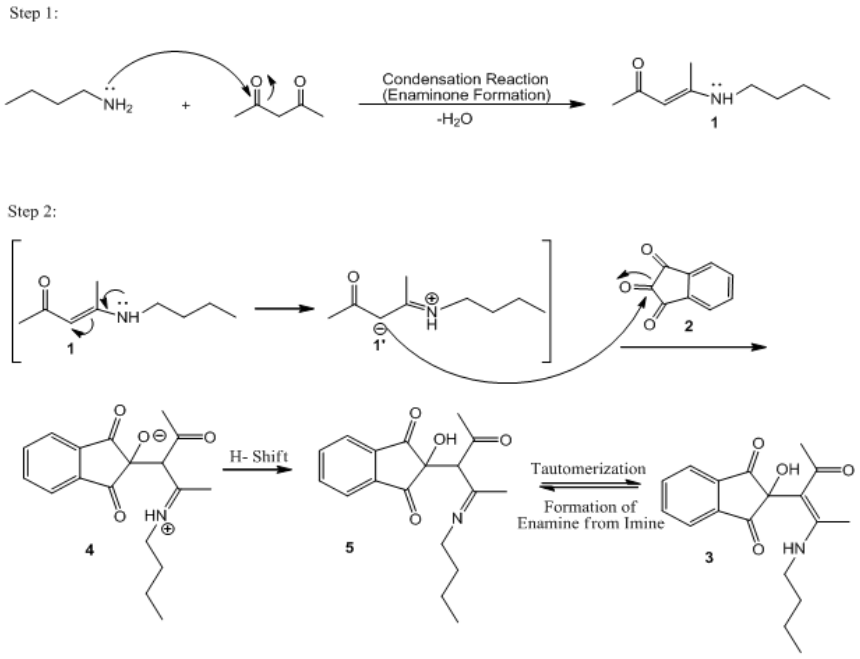
Our new synthetic method leading to the formation of the title compound is given in Scheme **1**. The reaction between butyl-amine and pentane-2,4-dione leads to Enaminone **1** through a known method[12]. After that by the addition of Nihydrin **2** the reaction proceeded gradually in methylethylketone (MEK) at ambient temperature and it was complete over 15 minutes to produce the *(Z)*-2-hydroxy-2-(4-oxo-3-(propylamino)pent-2-en-2-yl)-1H-indene-1,3(2H)-dione in excellent yield (Scheme 1).



Scheme 1: Synthesis of compound 3.

The structure of compound **3** was deduced from its elemental analysis, IR and high-field ^{1}H and ^{13}CNMR spectra and clearly indicated the formation of synthesized compound **3**. The mass spectrum of **3** displayed the molecular ion peak at m/z 315, which is in agreement with the proposed structure. The IR spectrum of this compound showed absorption bands due to NH group at 3515, a broad peak at 3473 for OH and three carbonyl groups were found at 1755, 1715, 1705 cm^{-1}. The ^{1}HNMR spectrum of **3** exhibited a triplet at δ_H 1.00 ppm ($^{3}J_{HH}$= 7.2 Hz) for methyl protons, two multiplets at δ_H 1.47 ppm, δ_H 1.55 ppm for two methylene groups and a broad s δ_H 1.75 ppm for OH, two sharp singlets at δ_H 2.36 ppm and δ_H 2.45 ppm for two methyl goups, a multiplet at δ_H 3.47 ppm for CH_2 linked to N and NH as a complex peak, along with multiplets at δ_H 7.56-7.89 ppm for the aromatic region. The ^{13}C NMR spectrum of 3 showed 14 distinct signals, which confirmed the proposed structure.

Although the mechanistic details of the reaction are not known, a plausible rationalization may be advanced to explain the product formation (Scheme 2). At the first step, the enaminone **1** formed from a condensation reaction between butan-1-amine and pentane-2,4-dione. Subsequently at the second step the reaction between enaminone **1** and 1H-indene-1,2,3-trione (Ninhydrine) **2** leads to zwitterionic intermediate **4.** Afterwards compound **5** is formed by an intermolecular proton shift. Finally, tautomerization of **5** leads to formation of **3**.



Scheme 2: Proposed mechanisms for the formation of 3.

1.8. Bioligical activity:

1.8.1. Cytotoxic evaluation:

In general the synthesized compound **3** in some concentrations was more active as compared to the standard drug, which may be taken as lead compound for the development of novel cytotoxic agent. The results of cytotoxic activity assessed by Cell viability assay (MTT) are presented in Table 1. Data generated were used to plot a dose–response curve of which the concentration of test compounds required to kill 50% of the cell population (IC_{50}) was determined. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments. The present study showed that the synthesized compound can be used as template for future development through modification and derivatization to design more potent and selective cytotoxic agent.

Table 1: Cytotoxic activity of synthesized compound 3 against C6 cell line at various concentrations Antimicrobial activity

Synthesized compound **3** Conce. (µg/ml)	500	250	125	62.5	31.25	15.625	7.812	3.906	1.953
OD 570 nm Triplicate	0.261	0.460	0.575	0.726	0.758	0.953	0.823	0.960	0.875
Control	0.258	0.582	0.524	0.685	0.788	0.972	0.706	0.896	0.888
	0.259	0.398	0.529	0.592	0.694	0.958	0.801	0.719	0.719
	0.783	0.789	0.767	0.777	0.769	0.787	0.780	0.757	0.788

Cytotoxicity%= (1- mean OD test/mean OD control) *10

The antimicrobial activity of the synthesized compound **3** was evaluated against a panel of 9 microorganisms and its potency was assessed qualitatively and quantitatively by the presence or absence of inhibition zones and MIC values. The disc diffusion method for antibacterial activity was showed significant reduction in bacterial growth in terms of zone of inhibition around the disc and the results are given in Table 2. Among bacterial strains tested, *Escherichia coli, Salmonella typhi* and *Listeria monocytogenes* were found to be more sensitive to the compound. Other bacterial forms were inhibited by synthesized compound. The zone of inhibition increased on increasing the concentration of this compound in disc. This was showed the concentration dependent activity.

The antibacterial activity of the synthesized compound **3** against the bacterial strains was showed high values of MIC (Table 2). Our findings showed that the aforementioned compound had interesting activity against both Gram-negative and Gram-positive bacteria. The compound proved to be active against 3 out of the 6 bacterial strains used and was particularly active against *Escherichia coli, Pseudomonas aeroginosa* and *Salmonella typhi* (MIC values of 32µg/ml for the first and 64µg/ml for the others, respectively). As for *Listeria monocytogenes* and *Kocuria varians* an MIC value of 128µg/ml was found, while *Bacillus pumilus* was the least affected with an MIC value of 256µg/ml. This compound showed an inhibiting activity on disease causing Gram-negative and Gram-positive bacteria, especially *Escherichia coli*.

Table 2. Antimicrobial activity of the synthesized compound 3

Microorganism	MIC of the synthesized compound **3**	MIC of reference[a]	Zone of Inhibition of the synthesized compound **3** in mm (Mean ± SD)	Zone of Inhibition of the reference mm (Mean ± SD)[b]
Bacillus pumilus	512	64	10.6 ± 1.1	16.3 ± 0.3
Escherichia coil	32	16	15.4 ± 0.5	16 ± 0
Kocuria varians	128	32	10.6 ± 1.5	17.6 ± 0.5
Listeria monocytogenes	128	16	14.5 ± 0.5	14.3 ± 0.5
Pseudomonas aeroginosa	64	8	13.6 ± 0.5	16.3 ± 0.1
Salmonella typhi	64	32	14.3 ± 0.3	21.3 ± 0.5
Aspergillus flavus	256	64	12.3 ± 1.1	19.3 ± 0.5
Candida glabrata	512	64	10.1 ± 0.3	19 ± 0
Aspergillus niger	128	32	10 ± 0.1	22 ± 0

[a]Ampicillin, Tetracycline and Fluconazole were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively.
[b]The values represent the mean of four experiments ± SD. Ampicillin, gentamicin and ketoconazole (10 µg/disc) were used as references for Gram-positive, Gram-negative bacteria and fungus, respectively.

2. Conclusion:

In conclusion, we have developed a rapid and efficient method for synthesis a medicinal compound that indicates good cytotoxicity and antimicrobial potency. This synthesis method involves several advantages including the simplicity of performance, good yields of products and relatively short reaction time. The synthesized compound can be used as a template for future development through modification and derivatization to design more potent and selective cytotoxic agent. Also the synthesized compound was also screened for in vitro antimicrobial activity by using the disc-diffusion method and determining the minimal inhibitory concentration (MIC) against Gram positive and Gram negative bacteria, and fungi. This compound inhibited the growth of Gram negative bacteria, being Escherichia coli the most susceptible one with MIC of 32 µg/mL. This is particularly interesting from a medical point of view because this microbial agent is responsible for severe

opportunistic infections. We also screened the antifungal activity of this compound and it showed mildly significant activity against fungal.

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