Antimicrobial, anti-inflammatory, and antinociceptive activities of triazole, pyrazole, oxadiazine, oxadiazole, and sugar hydrazone-5-nitroindoline-2-one derivatives and a study of their computational chemistry: part II

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Objective
The aim of this study (part II) is to evaluate the antibacterial, anti-inflammatory, and antinociceptive activities of a series of 1H-1,2,4-triazol-3-yl)phenylimino) (methylbenzyl)-5-nitroindolin-2-ones, 1H-pyrazole-1-carbonyl)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-ones, 3-(4-(1,3,4-oxadizine-6-one)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-ones, 1,3,4-oxadiazol-2-yl)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-ones and 4-(-1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino) sugar hydrazone derivatives (**1–13**) and, in addition, to investigate their computational chemistry.

Methods
The synthesized compounds in (part I) **1–9** were evaluated for their antibacterial and antifungal activities using different strains of Gram-positive bacteria (*Bacillus subtilis*), Gram-negative bacteria (*Pseudomonas aeruginosa)*, yeast (*Candida albicans*), and four mold fungi (*Fusarium solani*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, and *Phomopsis obscurans*). The anti-inflammatory and antinociceptive activities of compounds **1–13** were evaluated using a hot-plate test, acetic acid-induced writhing in mice, formalin-induced nociception, a tail immersion test, and carrageenan-induced hind paw edema. For computational chemistry, a semiempirical MNDO method (Modified Neglect of Differential Overlap is a semi-empirical method for the quantum calculation of molecular electronic structure in computational chemistry) associated with HyperChem professional 7.5 programs was adapted.

Results and conclusion
Compounds 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)] benzohydrazide (**3**) and 3-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-one (**9**) showed the highest antibacterial and antifungal activities compared with clotrimazole and sulfamethoxazole as reference drugs. In contrast, compounds ethyl 4-(5-nitro-2-oxoindolin-3-ylideneamino) benzoate (**1**), 3-(4-(3-methyl-5-oxo-4,5-dihydro-1H-pyrazole-1-carbonyl) phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-one (**8**), D-glucose-4-(-1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino) hydrazone derivative (**10**), and D-arabinose-4-(-1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino) hydrazone derivative (**12**) showed significantly high anti-inflammatory and antinociceptive activities when compared with indomethacin and morphine as reference drugs. From the computational chemistry compounds, ethyl 4-(5-nitro-2-oxoindolin-3-ylideneamino) benzoate (**1**), ethyl 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)] benzoate (**2**), and 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)] benzohydrazide (**3**) yielded the lowest values of total energy and heat of formation, and had higher stability than other molecules.

Keywords:
antibacterial and antifungal, anti-inflammatory, antinociceptive, computational chemistry, oxadiazole, pyrazoles, sugar hydrazones, triazoles

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Introduction

A large number of indoline-2,3-one have powerful antibacterial, antifungal, anti-inflammatory activities [1–3]. The importance of oxadiazole, pyrazole, and triazole derivatives as chemotherapeutic agents is well established and associated with potent biological activities such as antimicribial and anti-inflammatory activities [4–6].

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Table 1 In-vitro antimicrobial activity expressed as diameter of the growth-inhibitory zone of the tested compounds 2–13

	Activity expressed in mm of inhibition zone diameter						
Compounds	*Bacillus subtilis*	*Candida albicans*	*Pseudomonas aeruginosa*	*Phomopsis obscurans*	*Colletotrichum gloeosporioides*	*Fusarium solani*	*Aspergillus niger*
2	6.87	6.67	7.45	6.56	10.6	7.67	11.7
3	10.7	9.45	8.86	9.76	13.9	11.7	15.7
4	1.45	1.78	1.55	ND	1.56	1.54	1.87
5	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND	ND	ND
9	6.65	5.45	6.76	4.65	8.56	6.45	9.65
10	0.67	0.23	0.54	ND	ND	0.78	1.67
11	ND	ND	ND	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	ND	ND
13	ND	ND	ND	ND	ND	ND	ND
Clotrimazole	–	–	–	3.76	2.76	3.67	2.91
Sulfamethoxazole	3.87	2.80	4.98	–	–	–	–

ND, not detected.

Table 2 *In vitro*, antifungal activity of the tested compounds 3 and 9 against the strain of *Aspergillus niger* (zone of inhibition in mm)

Compounds	Concentration (μg/ml)	Inhibition zones (mm) ± SE	Spores count/ml	Myceliadry weight (mg) ± SE	Cell concentration ($OD_{610\,nm}$)
Control (medium)	0.0	0.0	542.9	28.9 ± 1.6	2.570
3	0.007	7.0 ± 1.4[a]	67.92	14.7 ± 1.0	0.7665
	0.07	16.0 ± 1.4	32.8	6.80 ± 1.4	0.347
	0.1	26.5 ± 2.1	0.87	0.27 ± 1.1	0.0321
9	0.007	4.5 ± 1.7	94.7	17.8 ± 1.0	3.213
	0.07	12.0 ± 0.0	42.7	9.65 ± 0.3	0.956
	0.1	15.5 ± 2.3	2.76	1.56 ± 0.4	0.254
Clotrimazole	0.1	2.54	231.8	11.8	1.45

OD, optical density.

In view of the biological activity, and as a continuation of our research work on the synthesis of 1,2,4 triazole, 1-H pyrazole, 1,3,4 oxadiazin, 1,3,4 oxadiazole, and sugar hydrazone -5-nitro-2-oxoindolin derivatives (part I), it was of interest to evaluate their effects as antibacterial, antifungal, anti-inflammatory, and antinociceptive agents in addition to studying their computational chemistry.

Subjects and methods

Antibacterial and antifungal assays

The antibacterial activities of the tested compounds **1–9** were determined using a cup plate method [7].The in-vitro antibacterial method was carried out using one bacterium (*Bacillus subtilis*), and four fungal strains (*Fusarium solani*, *Aspergillus niger*, *Colletotrichum gloeosporioides*, and *Phomopsis obscurans*) and yeast (*Candida albicans*) were used for the antibacterial assay.

Compounds **3** and **9** were assayed for their antifungal activity against *A. niger*; potato dextrose agar was used in this study. The agar cup (8 mm) diffusion method was utilized in this study [8]. A volume of 200 μl of each compound was dispensed into wells, bored in agar plates, freshly seeded with the tested microorganisms under aseptic conditions. The diameter of the clear zone was recorded after 3 days at 28°C. Clotrimazole and sulfamethoxazole were used as the standard antifungal and antibacterial agents as reference drugs, respectively. The inoculated plates were placed in an incubator at 30°C for 3 days. One milliliter of the product configuration was dispensed into the first series of sterile test tubes immediately before the test procedure was initiated. Starting with the second sterile test tube using broth medium appropriate for each microorganism, 1 : 2 dilutions developed for the product configuration to achieve a dilution series of 1 : 1, 1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32, 1 : 64, 1 : 128, 1 : 256, 1 : 512, and 1 : 024. A volume of 1.0 ml was left after removing 1.0 ml from the last test tube. A volume of 1.0 ml inoculum of each challenge suspension was introduced into each tube in the series for the product configuration, the dilution containing $\sim 5 \times 10^6$ CFUml of challenge microorganism. A positive control tube containing only the appropriate broth and inoculums was prepared for each of the challenge microorganisms.

Antifungal assay against *Aspergillus niger*

The antagonistic activities of compounds **3** and **9** against *A. niger* were assayed in potato dextrose broth medium. Erlenmeyer flasks containing 100 ml of potato dextrose broth medium containing 0.007, 0.07, and 0.1 μg/ml were inoculated with 2×10^3 spores of *A. niger* organism and incubated for 4 days on a rotary shaker (120 rpm) at 28°C. The growth of the tested yeast strains was estimated by measuring the optical density at 610 nm.

Antibacterial and antifungal activities

The results of antimicrobial activity were tabulated as inhibition zone diameter in millimeter from the pure compounds **2–13** as shown in Table 1. The tested

Table 3 Minimum inhibitory concentrations (μg/ml) of the tested compounds 3 and 9 against various microorganisms

	MIC (expressed as product dilution)						
Compounds	*Bacillus subtilis*	*Candida albicans*	*Pseudomonas aeruginosa*	*Phomopsis obscurans*	*Colletotrichum gloeosporioides*	*Fusarium solani*	*Aspergillus niger*
3	1 : 32	1 : 64	1 : 32	1 : 64	1 : 32	1 : 8	1 : 64
9	1 : 16	1 : 32	1 : 16	1 : 66	1 : 32	1 : 8	1 : 66
(Clotrimazole)	–	–	–	1 : 8	1 : 16	1 : 8	1 : 8
(Sulfamethoxazole)	1 : 8	1 : 16	1 : 8	–	–	–	–

MIC, minimum inhibitory concentration.

compounds showed high to moderate antibacterial activity against both gram-positive and gram-negative strains of bacteria and yeast at a concentration of 0.007 μg/ml. Compounds **3** and **9** showed the highest activity. It was also observed that compounds **3** and **9** showed activities against *Bacillus subtilis*, with an inhibition zone of diameter 10.7 and 6.65 mm, respectively. The antibacterial activity observed in this study was concentration dependent. The antifungal activity of the synthesized compounds was studied for the four pathogenic fungi at a concentration of 0.007 μg/ml. Some of the tested compounds showed weak to strong antifungal activity against all three strains of fungi. It was also observed that compounds **3** and **9** showed the highest activity (Table 2). This was observed strongly against *A. niger*, where an inhibition zone of diameter 15.7 and 9.65 mm, respectively, was observed. The antagonistic activity of compounds **3** and **9** against *A. niger* was assayed using three concentrations (0.007, 0.07, and 0.1 μg/ml). Ethyl-4(1-benzyl-5-nitro-2-oxoindalin-3-ylideneamino) benzohydrazide (**3**) was the most active compound that was antifungal. The growth of *A. niger* was strongly inhibited in the presence of compounds **3** than **9**, expressed as spores/ml, biomasses, growth rate per hour, and concentration.

Minimal inhibitory concentration measurement

The bacteriostatic activity of the active compounds **3** and **9** was then evaluated using the two-fold serial dilution technique [9,10]. Two-fold serial dilutions of the test compounds and reference drugs solutions were prepared using the proper nutrient broth. The final concentration of the solutions varied between 500 and 7.81 μg/ml, with the concentration of DMF not exceeding 2.5%. Each 0.10 ml from the tested compounds in DMF was mixed with 1, 2, and 3 ml of sterilized distilled water and 0.10 ml from each diluted samples was added to the test tubes. The tubes were then inoculated with the test organisms, grown in a suitable broth at 37°C for 24 h for bacteria and 48 h for fungi (about 1×10^6 cells/ml); each 5 ml received 0.10 ml of the above inoculum and were incubated at 37°C for 48 h. The minimum inhibitory concentration for compounds **3** and **9** was significantly higher than those of compound **9** as shown in Table 3.

Anti-inflammatory activity and antinociceptive activities

Animals

Male Swiss albino CD-1 mice (6–8 weeks old) were obtained from the Schistosome Biology Supply Center, Theodor Bilharz Research Institute (Giza, Egypt), and were housed under suitable laboratory conditions throughout the period of investigation. Animals were fed standard pellet chow (El-Nasr Chemical Company, Cairo, Egypt) and allowed free access to tap water.

Drugs and dosages

Morphine sulfate was administered intraperitoneally at a dose of 10 mg/kg (El-Nasr Pharmaceutical Co.).

Indomethacin was administered orally at a dose of 20 mg/kg (El-Kahira Pharmaceutical Co.).

Aspirin was administered orally at a dose of 100 mg/kg (Alexandria Pharmaceutical Co., Cairo, Egypt).

All tested compounds were suspended in 2% cremophore-El (Sigma Chemical Co., St. Louis, Missouri, USA) and administered orally at a dose of 200 mg/kg. This dose was chosen after determination of the LD_{50} of compounds **1–13** according to Litchfield and Wilcoxon [11].

Anti-inflammatory activity

Carrageenan-induced hind paw edema

A carrageenan-induced hind paw edema model was used for the determination of anti-inflammatory activity [12] 60 min after the oral administration of vehicle, indomethacin, and compounds **1–4** and **6–13**, and each mouse was injected with a freshly prepared (0.5 mg/25 μl) suspension of carrageenan in physiological saline (154 nmol/l NaCl) into subplantar tissue of the right hind paw. As a control, 25 μl saline solutions were injected into the left hind paw. Paw edema was measured every 1 h after the induction of inflammation. The difference in footpad thickness was measured using a plethysymometer 7150 (Ugo Basile, Como, Italy). The mean values of the treated groups were compared with the mean values of the control group and analyzed using statistical methods. Indomethacin (20 mg/kg) was used as a reference drug.

Antinociceptive activity

Acetic acid-induced writhing test in mice

Acetic acid (0.6% v/v, 10 ml/kg) was injected into the peritoneal cavities of mice, which were placed in a large glass cylinder, and the intensity of nociceptive behavior was quantified by counting the total number of writhes that occurred between 0 and 20 min after the stimulus injection, as described earlier [13]. Treatments with vehicle, indomethacin for compounds **1–4** and **6–13,** were administered 1 h before acetic acid injection ($n = 6$/group). Morphine sulfate was administered intraperitoneally

30 min before the test. The writhing response consists of a contraction of the abdominal muscle together with a stretching of the hind limbs. The antinociceptive activity was expressed as writhing scores over a period of 20 min.

Hot-plate test

The hot-plate test was used to measure the response of latencies according to the method described previously by Eddy and Leimback [14], with minor modifications. In this experiment, the hot plate (Ugo Basile; Model-DS37) was maintained at 55 ± 0.2°C. The reaction time was noted by observing either the licking of the hind paws or the jumping movements before and after drug administration. The cut-off time was 20 s and morphine sulfate 10 mg/kg (El-Nasr Pharmaceutical Co.) was administered intraperitoneally and used as a reference drug [15].

Formalin-induced nociception

A formalin solution (5% in 0.9% saline; 20 µl/paw) was injected into the hind paw plantar surface (intraperitoneally), and the animals were individually placed in transparent observation chambers, as described previously [16]. Oral treatments with vehicle, indomethacin, and compounds **1–4** and **6–13** were administered 1 h before formalin injection. Morphine sulfate was administrated (intraperitoneally) 30 min before the test. The time spent in licking the injected paw was recorded and expressed as the total licking time in the early phase (0–5 min) and the late phase (20–30 min) after formalin injection.

Tail immersion test

The lower two-thirds of the tail were immersed in a beaker containing water maintained at 50 ± 0.5°C [17]. The time (s) until the tail was withdrawn from the water was defined as the reaction time. The reaction time was measured at 0, 30, 60, and 120 min after the oral administration of vehicle, compounds **1–4** and **6–13** and morphine ($n = 6$/group), with the reaction time of 0 min being the start of the test. The mice were exposed to hot water for no longer than 20 s to avoid tissue injury.

Statistical analysis

The data obtained were analyzed using the Graph Pad software program Version 4.0 (Inc-La Jolla, California, USA) and expressed as mean ± SE. Statistically significant differences between groups were calculated using an analysis of variance, followed by the Newman–Keuls test. *P*-values less than 0.05 were considered as significant.

Results and discussion

Anti-inflammatory activity

No morbidity or mortality was recorded for any of the tested compounds **1–13**; LD_{50} was found to be 3000 mg/kg body weight as carrageenan-induced hind paw edema model was used for the determination of anti-inflammatory activity. After 1 h, the best inhibition was observed after the administration of compound ethyl 4-(5-nitro-2-oxoindolin-3-ylideneamino) benzoate (**1**) (53.92%) compared with the indomethacin reference drug

Table 4 Antinociceptive activity of the tested compounds *1–4* and *6–13* compared with the reference drug (aspirin) using the acetic acid-induced writhing test

Compounds	Dose of drug	Writhing number (count/20 min) (mean ± SE)	%Inhibition	%Potency
Control (acetic acid 0.7%/ saline)	0.01 ml/g	67.00 ± 1.69	–	–
1	200 mg/kg	20.83 ± 0.83*	68.91	90.83
2	200 mg/kg	40.50 ± 3.82*	39.55	52.13
3	200 mg/kg	32.33 ± 2.26*	51.74	68.21
4	200 mg/kg	33.50 ± 5.16*	50.00	65.91
6	200 mg/kg	28.67 ± 3.15*	57.20	75.41
7	200 mg/kg	26.83 ± 2.30*	59.95	79.02
8	200 mg/kg	22.67 ± 2.23*	66.16	87.21
9	200 mg/kg	28.83 ± 2.55*	56.97	76.08
10	200 mg/kg	23.33 ± 4.88*	65.18	85.91
11	200 mg/kg	44.67 ± 3.94*	33.34	43.93
12	200 mg/kg	22.00 ± 1.00*	67.16	90.16
13	200 mg/kg	26.83 ± 4.66*	59.96	79.03
Aspirin	100 mg/kg	16.17 ± 1.40*	75.86	100

*Significant difference from the control group at $P < 0.05$.

Table 5 Antinociceptive activity of the tested compounds *1–4* and *6–13* compared with reference drug (morphine) using a hot-plate test

Compounds	Dose of drug (mg/kg)	Reaction time (s) Mean ± SE	%Increase	%Potency
Normal control	Saline	11.00 ± 1.63	–	–
1	200	18.50 ± 0.67*	68.18	77.55
2	200	13.83 ± 0.87	25.73	39.60
3	200	14.83 ± 1.58	34.18	39.60
4	200	15.00 ± 1.00	36.36	41.36
6	200	16.17 ± 1.51*	47.00	53.46
7	200	16.17 ± 1.14*	47.00	53.46
8	200	17.00 ± 0.68*	54.54	62.04
9	200	15.67 ± 1.87	42.45	48.29
10	200	16.67 ± 1.09*	51.54	58.63
11	200	13.50 ± 2.29	22.72	25.85
12	200	16.67 ± 2.30*	51.54	58.63
13	200	16.00 ± 1.37*	45.45	44.77
Morphine	10	20.67 ± 1.74*	87.90	100

*Significant difference from the control group at $P < 0.05$.

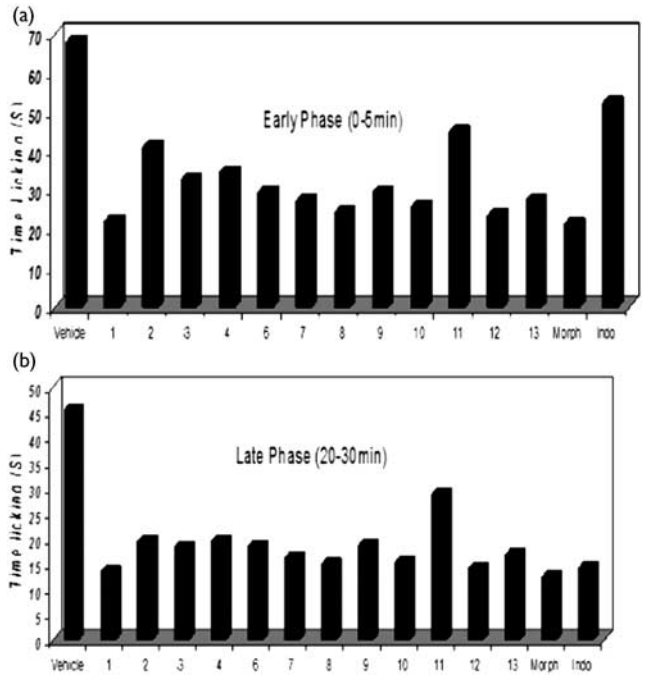
(50.66%), followed by compounds 3-(4-(3-methyl-5-oxo-4, 5-dihydro-1H-pyrazole-1-carbonyl) phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-one (**8**), D-glucose-4-(-1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino) hydrazone derivative (**10**), D-arabinose-4-(-1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino) hydrazone derivative (**12**) (about 35–37%), and then compounds 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)] benzohydrazide (**3**), 3-(4-(4-benzyl-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-one (**4**), 3-(4-(3,5-dimethyl-1H-pyrazole-1-carbonyl)-phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-one (**6**), 3-(4-(1,3,4-oxadiazin-6-one)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-one (**7**), 3-(4-(5-methyl-1,3,4-oxadiazol-2-yl)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-one (**9**), D-ribose-4-(-1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino) hydrazone derivative (**13**) (22–27%), whereas ethyl 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)]benzoate (**2**) and D-mannose-4-(-1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino) hydrazone derivative (**11**) showed weak effects (12.5,

Table 6 Anti-inflammatory activities of tested compounds 1–4 and 6–13 compared with the reference drug (indomethacin) using a carrageenan-induced hind paw edema model ($n=6$)

	%Edema at 1 h			%Edema at 2 h			%Edema at 3 h			%Edema at 4 h		
Compounds/dose (mg/kg)	Mean ± SE	%Inhibition	%Potency	Mean ± SE	%Inhibition	%Potency	Mean ± SE	%Inhibition	%Potency	Mean ± SE	%Inhibition	%Potency
Control Saline	26.67 ± 3.01	–	–	37.92 ± 2.40	–	–	47.69 ± 2.68	–	–	41.58 ± 2.80	–	–
1 200	14.16 ± 2.18*	**46.91**	**92.59**	17.68 ± 1.67*	53.38	95.61	20.75 ± 1.66*	56.49	95.74	15.49 ± 2.58*	62.74	94.59
2 200	23.50 ± 2.03	12.5	23.46	30.65 ± 2.34	19.17	34.34	36.12 ± 4.76	24.26	41.12	31.01 ± 5.64*	25.42	38.32
3 200	20.60 ± 2.62	22.75	44.93	26.81 ± 2.56*	29.30	52.48	27.58 ± 0.89*	42.17	71.46	20.67 ± 3.11*	50.28	75.82
4 200	20.74 ± 2.00	22.23	43.89	28.18 ± 2.31*	25.69	46.01	27.84 ± 4.36*	41.62	70.54	19.99 ± 4.76*	51.92	78.28
6 200	19.45 ± 1.82	27.07	53.44	27.10 ± 0.84*	28.53	51.11	27.72 ± 2.53*	41.87	70.97	23.56 ± 2.36*	43.33	65.34
7 200	19.80 ± 3.17	25.75	50.85	25.12 ± 3.10*	33.76	60.46	30.02 ± 4.63*	37.05	62.79	23.24 ± 3.47*	44.12	66.50
8 200	16.80 ± 2.57*	37.00	73.06	22.14 ± 2.41*	41.61	74.54	24.45 ± 2.18*	48.73	82.59	19.65 ± 2.03*	52.74	79.51
9 200	20.40 ± 1.90	23.50	46.41	27.63 ± 3.17*	27.14	48.61	29.69 ± 4.96*	37.74	63.97	21.51 ± 3.07*	48.26	72.77
10 200	17.27 ± 2.02*	35.24	69.58	21.74 ± 3.26*	42.66	76.43	22.57 ± 5.05*	52.67	89.27	17.80 ± 4.59*	57.19	86.22
11 200	22.07 ± 2.70	17.24	34.05	32.32 ± 3.78	14.76	26.45	38.09 ± 6.85	20.13	34.12	25.50 ± 1.71*	38.67	58.30
12 200	16.56 ± 3.73*	37.91	74.83	23.08 ± 1.64*	39.13	70.10	27.32 ± 1.95*	42.71	72.39	18.07 ± 3.40*	56.54	85.24
13 200	19.66 ± 2.14	26.28	51.89	26.90 ± 3.09*	29.06	52.05	30.57 ± 3.24*	35.89	60.84	20.63 ± 3.30*	50.38	75.96
Indomethacine 20	13.16 ± 1.44*	**50.66**	100	16.75 ± 1.70*	55.83	100	19.55 ± 1.77*	59.01	100	14.00 ± 1.63*	66.33	100

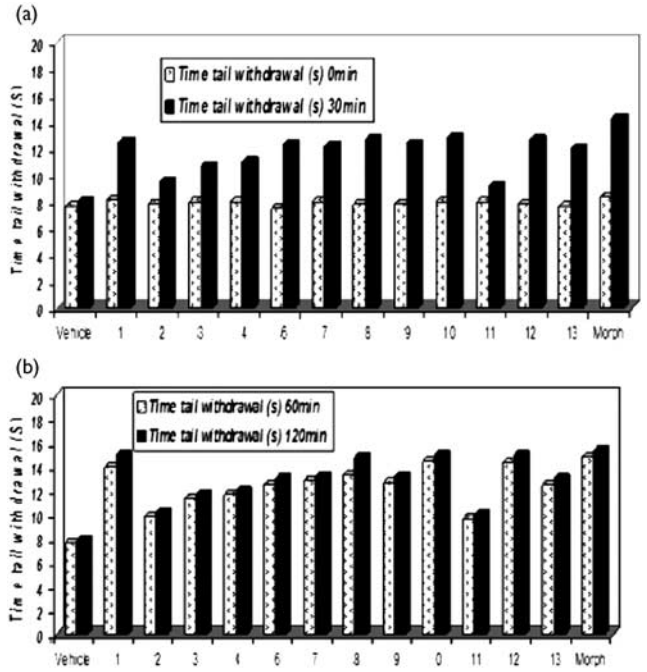
*Significant difference from the control group.

Figure 1



Effect of the synthesized compounds **1–13** administered orally (200 mg/kg) on licking induced by formalin in mice compared with indomethacin (20 mg/kg) or morphine (10 mg/kg) before formalin. The total time spent licking the hind paw was measured (a) in the early phase (0–5 min) and (b) the late phase (20–30 min) after an intraplanter injection of formalin. Each column represents the mean for six mice in each group.

Figure 2



Effect of the synthesized compounds **1–13** administered orally (200 mg/kg) on licking induced by formalin in mice compared with morphine (morph) (10 mg/kg) before tail immersion at 50°C. The time of tail withdrawal (s) was measured after (a) 0 and 30 min and after (b) 60 and 120 min. Each column represents the mean for six mice in each group.

17.24%). The highest effect of the anti-inflammatory activity of the tested compounds was observed after 4 h (Table 4). The percentage potency of the anti-inflammatory activity was measured and it was found that compound ethyl 4-(5-nitro-2-oxoindolin-3-ylideneamino) benzoate (**1**) had the highest potency (>92%) compared with the reference drug indomethacin.

Antinociceptive activity

The total number of writhings produced 20 min after the intraperitoneal injection of acetic acid control was about 67.00 ± 1.69. Reduction in the number of writings was found for ethyl 4-(5-nitro-2-oxoindolin-3-ylideneamino) benzoate (**1**) (68.91%), then compound **13** > **7** > **6** > **9**, followed by **3** > **4**, whereas the least reduction was shown by compound **2** (33%) and **11** (39%).

In the hot-plate test (Table 6), compound ethyl 4-(5-nitro-2-oxoindolin-3-ylideneamino) benzoate (**1**) showed increase in the latency time as compared with the control group ($P<0.05$). The % analgesia of **1** was found to be 77.55% when compared with the reference drug (morphine).

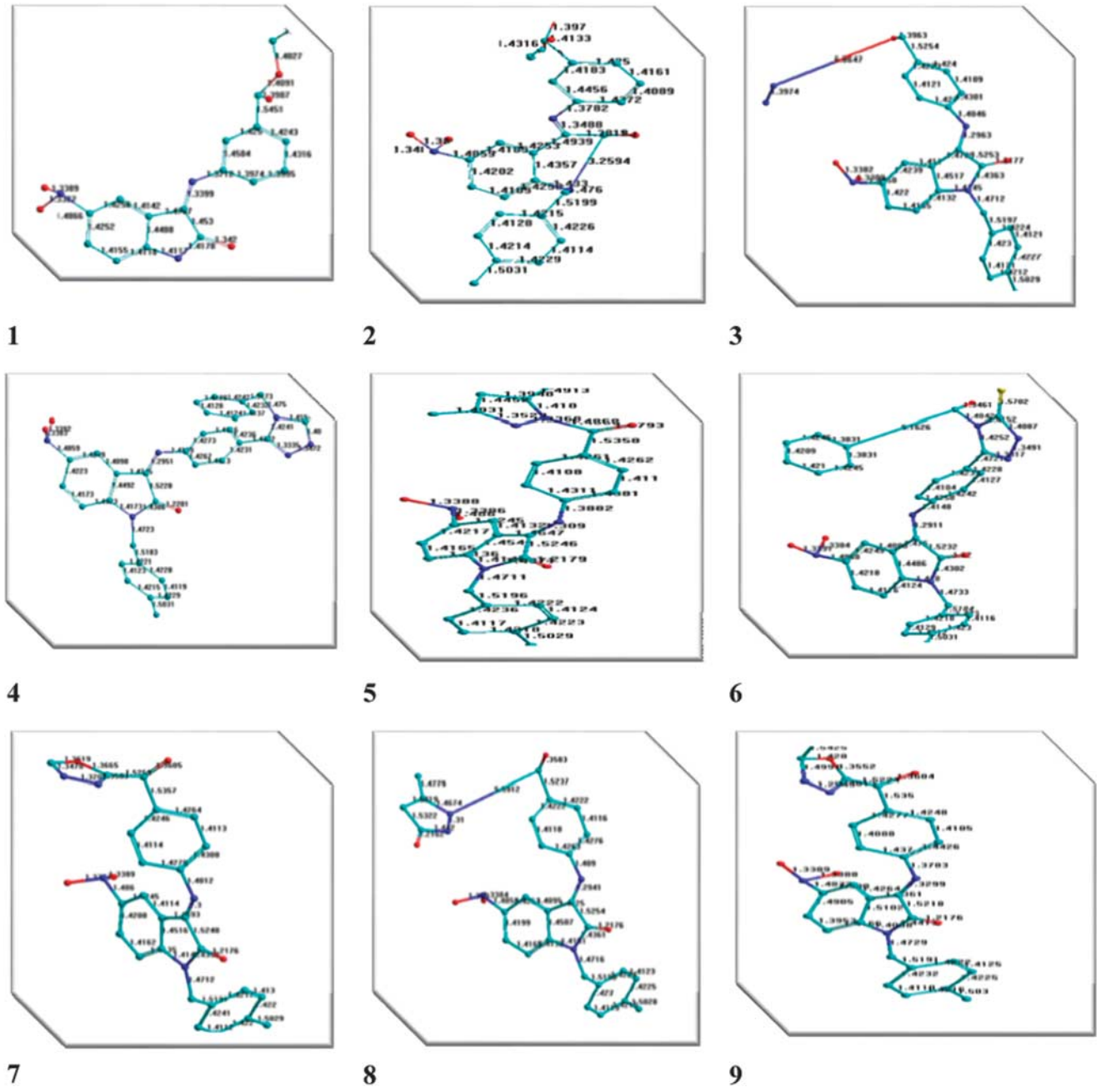
In the tail immersions test shown in Fig. 1, an increase in the tail-flick response latency time was recorded for compound **1** (91.57%), followed by **8** > **12** > **10** > **7** > **9** > **6** > **13** > **4** > **3** as compared with the control group ($P<0.05$), either after 60 or 120 min from drug administration. The least antinociceptive effect was recorded for ethyl 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)] benzoate (**2**) (29%) and D-mannose-4-(-1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino) hydrazone derivative (**11**) (27%) when compared with the reference drug (morphine).

Figure 2 shows that compound **1** had the best anti-nociceptive activity compared with the control group in both the early and the late phase ($P<0.05$).

Computational chemistry study

HyperChem professional 7.5 programs [18] procedure was used and compared with the experimental data. The results were investigated through regression and correlation analysis, after optimization of geometries, to calculate the thermochemical values for the synthesized compounds **1–9** (part I) and their bond lengths using the semiempirical molecular orbital procedure MNDO [19] (Table 7). Use of the semiempirical method for these types of calculations provides considerable insights into the structure and reactivity of such molecules [20,21]. Different transition structures and reactive pathways were obtained in Fig. 3. From Table 7, we found that compound ethyl 4-(5-nitro-2-oxoindolin-3-ylideneamino) benzoate (**1**), 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)] benzoate (**2**) and 3 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)] benzohydrazide (**3**) had the lowest values of total energy and heat of formation respectively, which means that they have higher stability than the other molecules **4**, **5**, **6**, **7**, **8**, **and 9**, with higher values of heat of formation. Compound 4-[(1-(*p*-methylbenzyl)-5-nitro-2-oxoindolin-3-ylideneamino)] benzohydrazide (**3**) is the most stable of the other tested compounds.

Figure 3



Geometry optimization of the studied molecules using the MNDO method (with bond length values).

Table 7 Total energy, dipole moment, and heat of formation for tested compounds 1–9 after optimization using the MNDO method

	Compounds								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Total energy (kcal/mol)	−107 042	−133 588	−129 503	−155 987	−163 332	−148 034	−149 220	−157 457	−153 125
Dipole moment	1.834	4.633	5.912	8.577	9.184	5.513	6.895	5.491	5.887
Heat of formation (kcal/mol)	−40	−48	−11	103	148	127	95	45	121

Conclusion

A series of aromatic heterocyclic compounds containing 1H-1,2,4-triazol-3-yl)phenylimino)(methylbenzyl)-5-nitroindolin-2-ones, 1H-pyrazole-1-carbonyl)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-ones, 3-(4-(1,3,4-oxadiazin-6-one)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-ones, 1,3,4-oxadiazol-2-yl)phenylimino)-1-(*p*-methylbenzyl)-5-nitroindolin-2-ones, 4-(-1-(*p*-methylbenzyl)-5-nitro-2-ox-

oindolin-3-ylideneamino) sugar hydrazone derivatives (part1) were evaluated for their antibacterial, antifungal, anti-inflammatory, and antinociceptive activities. It was found that compounds **3** and **9** were the most active in terms of antibacterial and antifungal activities, with *para*-methyl substitution at the benzyl ring and *para*-hydrazide moiety or the oxadiazol-2-yl ring, in addition to the nitro substitution in the 5-position of oxoindoline-2-one, enhancing the antibacterial and antifungal properties. Compounds **1**, **8**, **10**, and **12** showed significant anti-inflammatory and antinociceptive activities compared with the reference drugs. Using the semiempirical molecular orbital procedure MNDO, compounds **1**, **2**, and **3** were found to be the most stable compounds.

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Conflicts of interest

There are no conflicts of interest.

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