Synthesis, Characterization, and Screening for Anti-inflammatory and Antimicrobial Activity of Novel Indolyl Chalcone Derivatives

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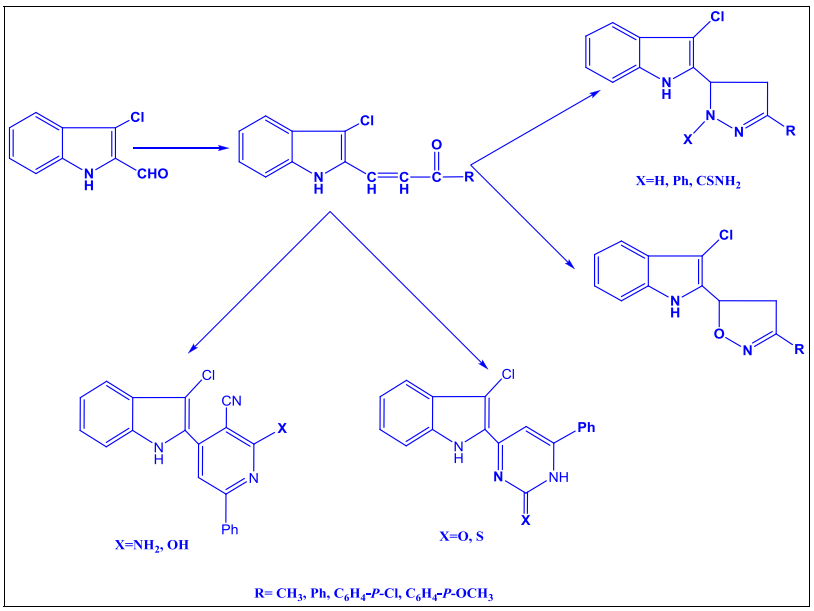


Because of the great biological importance of substituted indole derivatives, in the present study, a series of pyrazolylindole, thiazolylindole, and pyrimidinylindole derivatives have been synthesized with good yield. The precursor indolyl chalcone **2a**–**d** was prepared by reaction of 3-chloro-1*H*-indole-2-carbaldehyde **1** with different ketones. Then, compounds **3b**–**d**, **4**, and **5a**–**d** have been synthesized by the reaction of chalcones **2a**–**d** with hydrazine, phenylhydrazine, and thiosemicarbazide. When the chalcone derivative **2b** subjected to react with hydroxylamine hydrochloride gave isoxazolylindole derivative **6b**. *N*-thiazolidine pyrazolyl indole **7** was obtained by reacting compound **5a** with ethyl chloroacetate. On the other hand, when chalcone derivative **2b** allowed to react with urea and thiourea gave the corresponding pyrimidinylindole derivatives **8** and **9**. Finally, when chalcone derivative **2b** reacted with ethyl cyanoacetate or malononitrile gave pyridinylindole derivatives **10** and **11**. The structures of the all synthesized compounds were elucidated on the basis of spectral analysis infrared, NMR, and mass spectroscopy. Some of the synthesized compounds were screened for their antimicrobial and anti-inflammatory activity. Compound **4b** was the highest antibacterial activity against all strains of bacteria with values higher than those of the corresponding reference antibiotics (ciprofloxacin and levofoxacin, respectively) and almost the same as (gemifloxacin, moxifloxacin, clindamycin, gentamycin, and streptomycin). Compounds **4**, **5**, **6**, and **7** showed high anti-inflammatory activity compared with the standard drug indomethacin.

INTRODUCTION

Heterocyclic compounds containing nitrogen have been described for their biological activity against various microorganisms. The indole unit is the key building block for a variety of compounds, which have crucial roles in the functions of biologically important molecules [1,2] Introduction of different groups to the modified indole structure can produce a series of compounds with multiple activities. Various 3-substituted indoles had been used as starting materials for the synthesis of a number of alkaloids, agrochemicals, pharmaceuticals, and perfumes. Also, 3-substituted indole derivatives possess various types of broad spectrum's biological activities such as antimicrobial, antitumor, hypoglycemic, anti-inflammatory, analgesic, and antipyretic activities [3,4]. Moreover, the substitution at the 3-position of the indole ring can take place by introducing an additional heterocyclic ring, such as imidazole (topsentins, nortopsentins) [5,6], dihydroimidazole (discodermindole)

[7], oxazole [8,9], thiazole [10], quinazoline [11], pyrimidine [12,13] as shown in Figure 1, and pyrazoline [14]. Therefore, 3-substituted indoles still represent a significant synthetic challenge. In view of the important biological properties of the indole ring, we have planned to synthesize a new series of 3-chloro-2-substituted indole derivatives bearing side chains with different structures, as such derivatives could possess interesting and useful antimicrobial and anti-inflammatory activities.

RESULTS AND DISCUSSION

Chalcone derivatives **2a–d** were synthesized by the reaction of chloro-aldehyde **1** using different ketones, namely, acetone, acetophenone, *p*-chloroacetophenone, and *p*-methoxyacetophenone in the presence of catalytic amount of 40% sodium hydroxide. The structures of chalcone derivatives were elucidated by spectral analyses, where their infrared (IR) spectra showed disappearance of band characteristic of C═O aldehydic, which existing in the starting material, and showed also appearance of new absorption band characteristic of C═O of chalcone at 1653 cm^{-1} for compound **2b** and 1647 cm^{-1} for compound **2d**; also, their ^1H NMR spectra showed appearance of new two doublet signals characteristic of CH═CH at δ 7.75, 8.01 ppm for compound **2b**, and δ 7.70 and 7.95 ppm for compound **2d**. When compound **2b** was treated with hydrazine hydrate, phenylhydrazine and thiosemicarbzide in ethanol gave pyrazolyl indole derivatives **3b**–**5b**. The structures of these obtained compounds were confirmed by elemental and spectral analyses where IR spectrum of compound **3b** showed disappearance of band for carbonyl group, and ^1H NMR spectrum showed appearance of doublet signal at δ 2.66 ppm characteristic of Ha, doublet signal at δ 2.92 ppm characteristic of Hb, and triplet signal at δ 5.2 ppm characteristic of Hc, while the ^1H NMR spectrum of compound **5b** showed appearance of singlet signals at δ 2.98 ppm characteristic of Ha, 3.07 ppm characteristic of Hb, 6.79 ppm characteristic of NH_2, and 11.5 ppm characteristic of NH. When compound **2a** was refluxed with thiosemicarbazide in acetic acid, compound **5a** was obtained, then allowed to react with ethyl chloroacetate and fused sodium acetate in the presence of pyridine to afford compound **7**. The structure of compound **5a** was proved by IR spectrum, which showed appearance of new bands at 3177, 3264, 3371 cm^{-1} characteristics of NH and NH_2, while structure of compound **7** was elucidated by spectral analyses where IR spectrum showed appearance of band at 1719 cm^{-1} characteristic of C═O (Scheme 1).

Pyrimidinyl indole derivatives **8** and **9** were obtained by treating compound **2b** with urea or thiourea in the presence

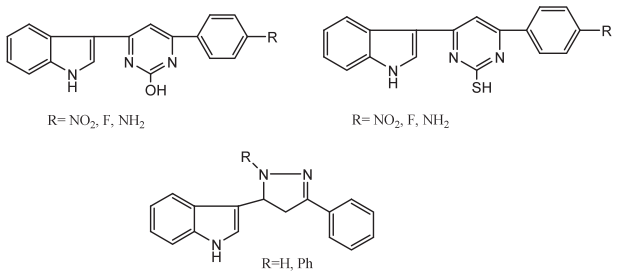
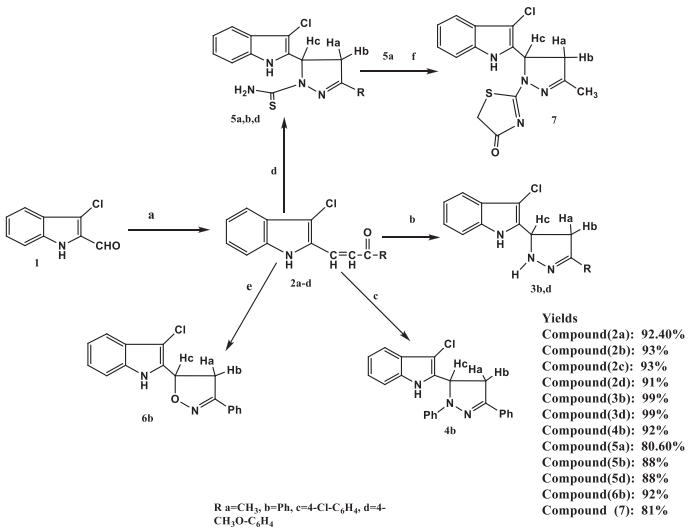


Figure 1. Previously reported compounds containing pyrimidinyl and pyrazolylindole as bioactive molecules [12].

Scheme 1. Synthesis of pyrazolyl and oxazolylindole derivatives.

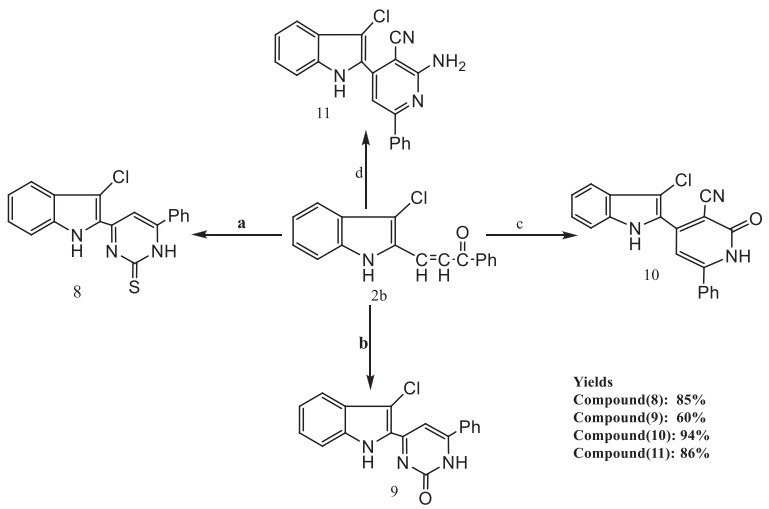


Scheme 1 Reagents and conditions: (a) $PhCOCH_3$, NaOH stirring 1h;(b)Hydrazine Hydrate, EtOH, Reflux 2 h; (c) $PhNHNH_2$, EtOH, Reflux 4h;(d)$NH_2CSNHNH_2$, EtOH, Reflux 1h;(e) NH2OH, CH_3COONa, EtOH (f) $ClCH_2COOEt$, CH_3COONa, EtOH

of sodium hydroxide in ethanol. The structures of the pyrimidine derivatives were elucidated by elemental and spectral analyses, where IR spectrum of compound **8** showed appearance of new band at 1232 cm^{-1} characteristic of C═S and disappearance of band characteristic of C═O in the starting material, ^1H NMR spectrum showed appearance of new singlet signals at δ 8.12 ppm and δ 11.30 ppm characteristic of CH pyrimidine and SH group, IR spectrum of compound **9** showed appearance of band at 3510 cm^{-1} characteristic of OH, and ^1H NMR spectrum showed appearance of signal at δ 8.12 ppm characteristic of OH. When compound **2b** was treated with ethyl cyanoacetate or malononitrile in ethanol in the presence of excess of ammonium acetate gave pyridinyl indole derivatives **10** and **11**. The structures of the obtained compounds elucidated by spectral analyses where IR spectrum of compound **10** showed appearance of new bands at 171 and 2215 cm^{-1} characteristic of C═O and CN groups, ^1H NMR spectrum showed appearance of singlet signal at δ = 8.18 ppm characteristic of CH pyrimidine, while IR spectrum of compound **11** showed appearance of new bands at 3205, 3315, and 2209 cm^{-1} characteristic of NH, NH_2, and CN group, ^1H NMR spectrum showed appearance of singlet signal at δ 4.52 ppm characteristic of NH_2 (Scheme 2).

Biological activities. ***Antimicrobial activity.*** Table 1 shows that all the investigated compounds presented remarkable antibacterial properties against Gram-positive bacteria, and compound **4b** is the highest antibacterial activity against all strains of bacteria, with values higher than those of the corresponding reference antibiotics (ciprofloxacin and levofloxacin, respectively) and almost the same as (gemifloxacin, moxifloxacin, clindamycin, gentamycin, and streptomycin), while compound **5b** showed low antibacterial activity compared with other tested compounds. Furthermore, compounds **5b**, **6b**, **7**, and **11** displayed excellent activity against other bacterial strains *Staphylococcus aureus* and *Bacillus cereus*. The inhibition of *Aspergillus niger*, *Pseudomonas*, and *Haemophilus influenza* was achieved by compounds **7** and **10**. As concerns, compound **5b** showed significant activity against all tested bacteria. In conclusion, the data in Table 1 showed that compound **4b**, which has the indole and pyrazole nucleus, has the highest antibacterial activity, while compound **10**, which has only indole moiety, has low activity against Gram-positive bacteria and moderate activity against Gram-negative bacteria, which mean that pyrazole moiety has remarkable activity against all strains of bacteria; compound **10** showed low activity against the all strains of bacteria except *A. niger* and *H. influenza*; the antifungal results for compounds against six fungal species were summarized in Table 2. From Tables 1 and 2, it could be stated that all the investigated compounds presented remarkable antifungal properties against six types of fungi *Penicillium sp*, *Geotrichum candidum*, *Candida albicans*, *Aspergillus fumigatus*, and *Syncephalastrum racemosum*. Table 2 showed that compound **10**, which has the indole and pyridine nucleus with cynao group in pyridine ring, has the highest antifungal activity against all the strains of fungi; from Tables 1 and 2, it could be stated that compound **4b**, which contains indolylpyrazolo moiety, has an antibacterial activity against all strains of Gram-positive and Gram-negative bacteria, with values higher than those of the corresponding standers (levofloxacin and ciprofloxacin), and has high antifungal activity against *S. racemosum*; on the other hand, compound **8** has

Scheme 2. Synthesis of pyridinyl and pyrimidinyl indole derivatives.



Scheme 2 Reagents and conditions: (a) NH_2CSNH_2, NaOH, Reflux 5 h;(b) NH_2CONH_2, Reflux 5h;(c) $CNCH_2COOET$, CH_3COONH_4, AcOH, Reflux 4h;(d)$CH_2(CN)_2$, CH_3COONH_4, AcOH, reflux 4h

Table 1

Antibacterial activity of the tested compounds (mm).

Bacteria								
Compound	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Bacillus cereus*	*Bacillus subtilis*	*Haemophilus influenza*	*Escherichia coli*	*Aspergillus niger*	*Pseudomonas sp*
3	26	20	25	24	26	12	26	17
4b	27	25	27	24	19	20	27	28
5b	22	18	22	26	16	26	27	22
6b	24	17	24	13	14	25	22	15
7	24	19	24	23	16	24	22	15
8	17	13	13	22	19	25	27	29
9b	9	10	18	15	21	25	26	24
10b	11	15	11	14	18	22	27	17
11	28	12	27	15	13	11	12	28
Ref.	25	20	28	25	20	26	28	30
	Ciprofloxacin	Levofoxacin	Moxifloxacin	Gemifloxacin	Clindamycin	Streptomycin	Gentamycin	Clindamycin

The amount added in each pore is 50 μL. (+) indicates Gram-positive bacteria, and (−) indicates Gram-negative bacteria (inhibition zone, mm).

Table 2

Antifungal activity (inhibition zone, mm).

Fungi						
Compound	*Penicillium sp*	*Candida albicans*	*Geotrichum candidum*	*Aspergillus fumigatus*	*Syncephalastrum racemosum*	*Geotrichum candidum*
3	14	18	19	17	13	15
4b	19	14	14	16	17	17
5b	15	19	18	14	19	12
6b	12	17	14	22	12	14
7	17	18	16	15	13	17
8	18	10	14	18	16	17
9b	17	20	17	21	17	22
10b	16	11	14	14	19	15
11	15	19	17	16	16	16
Ref.	18	21	18	22	19	23
Ketoconazole						

The amount added in each pore is 50 μL.

antifungal activity as the same as the stander *ketoconazole* against *Penicillium sp*, whereas compound **10** has the lowest activity against the same fungal strain; this means that this type of fungi is sensitive to the pyrimidine nucleus in compound **8**. On the other hand, compounds **4b**, **5b**, **8**, and **10** showed high antifungal activity against *Penicillium sp*, *G. candidum*, and *S. racemosum*, while compound **6b** showed the highest antifungal activity against *A. fumigatus*. Also, compound **9** showed the highest antifungal activity against *C. albicans*. However, compounds **3b**, **7**, **8**, and **10** showed moderate activity.

Anti-inflammatory evaluation. The results of anti-inflammatory activity are summarized in Tables 3 and 4 and Fig. 2. From the data presented in Tables 3 and 4, the tested compounds, **3b**, **4b**, **5b**, **6b**, and **7**, were found to be active and have significant activity (22.71%, 43.17%, 40.91%, 36.35%, 43.17% and 22.22%, 26.67%, 35.56%, 26.67%, 31.10% inhibition in paw edema at 4 and 5 h, respectively) when compared with the standard drug indomethacin (47.72% and 42.22% at 4 and 5 h, respectively). Compounds **4b**, **5b**, **6b**, and **7** showed high anti-inflammatory activity compared with the standard drug indomethacin. Where the size of edema is the same at 0 time and after 30 min has no significant difference in the size of edema, but a significant difference exists between indomethacin and compound **3b**, which has *P* value less than 0.05 that means compound **3b** does not effect on inflammation of rats after 30 min. After 1 h, most compounds showed significant differences from indomethacin like compounds **4b**, **6b**, and **7**, where compound 3-chloro-2-(1,3-diphenyl-4,5-dihydro-1*H*-pyrazol-5-yl)-1*H*-indole (**4b**) showed the highest activity against the paw edema inflammatory due to the existing of the active pyrazole ring, but after 2 h, the tested compounds show significant activity higher than that at the first hour, and compounds **4b** and **5b** have the same highest activity (31.10%) among all the tested compounds, which is very close to the activity of the reference (33.33%); also, the activity of compound **3b** became high with increasing the time. After 3 h, the activity of all the tested compounds becomes more higher, and compounds **4b** and **5b**, which have the pyrazole ring with indole moiety, still have the greatest activity in addition to compound **7**, which has the same activity as compounds

Table 3

Anti-inflammatory activity of tested compounds (**3**, **4b**, **5b**, **6b**, **7**) using acute carrageenan-induced paw edema in rats (statistical analysis).

	Paw edema inhibition (mean ± SEM)[a,b,c,d](%)					
Compound[a] Time	30 min	1 h	2 h	3 h	4 h	5 h
3	0.6833 ± 0.01667	0.6833 ± 0.01667	0.6333 ± 0.01667	0.6167 ± 0.03333	0.5667 ± 0.01667	0.5 ± 0.01
4b	0.6500 ± 0.02887	0.5500 ± 0.02887	0.5167 ± 0.04410	0.4667 ± 0.01667	0.4167 ± 0.01667	0.5500 ± 0.02887
5b	0.6833 ± 0.01667	0.6333 ± 0.01667	0.5167 ± 0.01667	0.4667 ± 0.01667	0.4333 ± 0.01667	0.4833 ± 0.01667
6b	0.6833 ± 0.01667	0.6000 ± 0.02887	0.5333 ± 0.04410	0.5000 ± 0.02887	0.4667 ± 0.01667	0.5500 ± 0.02887
7	0.7000 ± 0.0	0.6000 ± 0.02887	0.5333 ± 0.03333	0.4667 ± 0.01667	0.4167 ± 0.01667	0.5167 ± 0.01667
Indomethacin	0.6500 ± 0.02887	0.5833 ± 0.01667	0.5000 ± 0.05774	0.4000 ± 0.0	0.3833 ± 0.01667	0.4333 ± 0.01667
Control	0.7333 ± 0.01667	0.7333 ± 0.01667	0.7500 ± 0.0	0.7667 ± 0.01667	0.7333 ± 0.03333	0.7500 ± 0.02887

SEM, standard error of the mean.
[a]Dose 20 μmol/kg.
[b]$n = 6$.
[c]Statistically significant from the indomethacin at $P < 0.05$.
[d]Potency was expressed as % edema inhibition of the tested compounds relative to % edema inhibition of indomethacin (reference drug).

Table 4

Paw edema inhibition (%).

Time	Anti-inflammatory activity (% inhibition)					
Compound[a]	30 min	60	120	180	240	300
3	6.81	6.81	15.65	19.56	22.71	22.22
4b	11.35	24.99	31.10	39.12	43.17	26.67
5b	6.81	13.63	31.10	39.12	40.91	35.56
6b	6.81	18.17	28.89	34.78	36.35	26.67
7	4.54	18.17	28.89	39.12	43.17	31.10
Indomethacin	11.35	20.45	33.33	47.82	47.72	42.22

Mean increase in paw volume in rats treated with tested compounds and mean increase in paw volume in the control group of rats.
[a]Dose 20 μmol/kg.

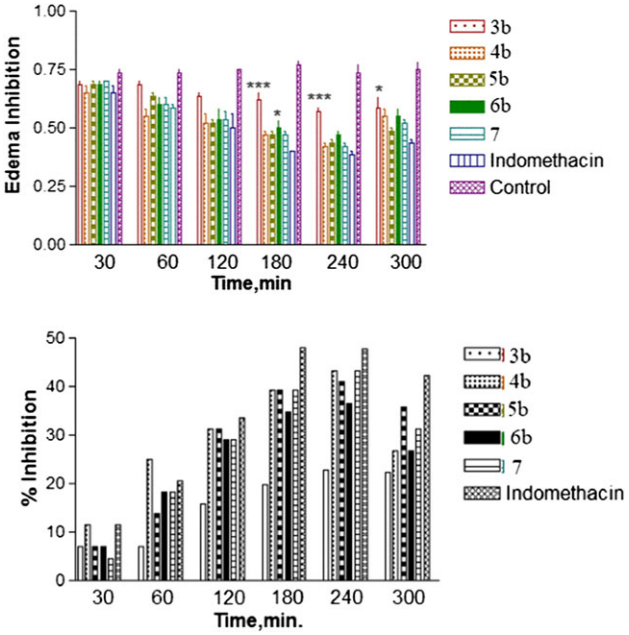


Figure 2. Paw edema inhibition (%) with the time in mins. [Color figure can be viewed at wileyonlinelibrary.com]

5b and **6b**. Also, compound **7b** shows significant activity relative to the indomethacin, while compound **3b** has $P < 0.05$, which means that compound **3b** has low significance after 3 h; also, compound **3b** after 4 h showed low significant difference from the reference compound ($P < 0.001$). After 5 h, compounds **4b**, **5b**, **6b**, and **7** showed no significance from indomethacin, and compound **6** has the highest activity, while compound **3b** showed low significance ($P < 0.05$).

CONCLUSION

The objective of the present study was to synthesize, characterize, and investigate antimicrobial and anti-inflammatory activities of some new indolyl chalcones and their derivatives starting from compound 3-chloro-1*H*-indole-2-carbaldehyde to give the target compounds. Compounds **4b**, **5b**, **6b**, and **7** found to be the most active compounds against all species of fungi and bacteria.

EXPERIMENTAL

Chemistry. All melting points are uncorrected and measured on a Fisher-John apparatus (Jacobs University, Bremen, Germany). Elemental analyses were determined on an Elementar Analysen system GmbH-VarioEL V2.3 micro-analyzer in the central lab of Assiut University. Their results were found to be in good agreement (±0.2%) with the calculated values. IR spectra were recorded on a Pye-Unicam Sp-100 spectrophotometer using KBr wafer technique, and values were represented in cm^{-1}. ^1H NMR and ^{13}C NMR were carried out on Varian Gemini 300 MHz spectrophotometer at the Microanalytical Center, Cairo University, Cairo, Egypt, and Bruker 400 MHz spectrophotometer at Jacobs University, Germany using tetramethylsilane as internal standard in deuterated dimethyl sulfoxide (DMSO-d_6) and deuterated chloroform ($CDCl_3$), and the chemical shifts were recorded in ppm δ scale. The electron impact mass spectra were recorded on JEOL JMS-600 spectrometer at the Central Unit for Analysis and Scientific Service, National Research Center, Cairo, Egypt. Analytical thin-layer chromatography were carried out on silica gel plates (Fluka 70643-50EA, Sigma-Aldrich, Germany) using ultraviolet light. All reactions were carried out under an air atmosphere. 3-chloro-1*H*-indole-2-carbaldehyde (**1**) was synthesized according to literature procedure [15] with mp 174–175°C.

General procedure for the synthesis of chalcone derivatives (2a–d). A solution of compound (I) (1 gm, 5.57 mmol) in ethanol and selected ketone (5.57 mmol) was stirred at 10°C for 2 h in the presence of sodium hydroxide (40%). The solid product formed during stirring was filtered off, dried, and recrystallized from the proper solvent.

4-(3-Chloro-1H-indol-2-yl)but-3-en-2-one (2a). The compound obtained was recrystallized from ethanol/water as a yellow crystal in 92.40% yield, mp 181–183°C. IR (KBr): cm^{-1} 3050 (CH aromatic), 1659 (C═O), 3290 (NH). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.32 (s, 3H, CH_3), 6.67–7.67 (m, 4H, Ar─H), 7.83 (d, 1H, CH), 8.04 (d, 1H, CH), 12.48 (s, 1H, NH) ppm. ^{13}C NMR (100 MHz, DMSO-d_6) (ppm) 26.72, 112.44, 114.49, 119.52, 121.30, 124.46, 124.60, 127.72, 131.80, 132.83, 136.43, 198.75. *Anal*. Calcd for $C_{12}H_{10}ClNO$ (219.05): C, 65.61; H, 4.59; Cl, 16.14; N, 6.38%. Found: C, 65.23.13; H, 4.68; Cl, 16.42; N, 4.45%.

2(3-Chloro-1H-indol-2-yl)-1-phenylprop-2-en-1-one (2b). The compound obtained was recrystallized from ethanol as a yellow crystal in 93% yield, mp 190–192°C. IR (KBr): cm^{-1} 3050 (CH aromatic), 1653 (C═O), 3285 (NH). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.13–7.90 (m, 9H, Ar─H), 6.36 (d, 2H, CH), 7.81 (d, 2H, CH), 11.28 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 109.43, 111.42, 112.96, 119.01, 121.45, 122.63, 125.05, 126.25, 129.04, 129.43, 130.66, 133.47, 136.63, 138.24, 188.82. MS *m/z* (M^+, 281.08). *Anal*. Calcd for $C_{17}H_{12}ClNO$ (281.06): C, 72.47; H, 4.29; Cl, 12.58; N 4.97%. Found: C, 72.03; H, 4.43; Cl, 12.68; N, 4.93%.

3-(3-Chloro-1H-indol-2-yl)-1-(4-chlorophenyl)prop-2-en-1-one (2c). The compound obtained was recrystallized from ethanol/dioxane as a yellow crystal in 93% yield, mp 220–223°C. IR (KBr): cm^{-1} 3050 (CH aromatic), 1697 (C═O), 3281 (NH). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 6.25 (d, 1H, CH), 7.01 (d, 1H, CH),

7.60–8.20 (m, 8H, Ar—H), 11.50 (s, 1H, NH) ppm. MS: *m/z* (M^+, 205.70). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 112.44, 114.49, 119.52, 121.30, 124.43, 124.60, 129.12, 129.69, 131.80, 132.83, 136.43, 137.35, 138.69, 191.43. *Anal*. Calcd for $C_{17}H_{11}Cl_2NO$ (315.02): C, 64.58; H, 3.51; Cl, 22.43; N 4.43%. Found: C, 64.09.13; H, 3.60; Cl, 22.40; N, 4.48%.

3-(3-Chloro-1H-indol-2-yl)-1-(4-methoxyphenyl)prop-2-en-1-one (2d). The product obtained was recrystallized from dioxane as yellow crystals in 91% yield, mp 310–311°C. IR (KBr): cm^{-1} 3050 (CH aromatic), 1647 (C═O), 3281 (NH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.88 (s, 3H, CH_3) 7.13–7.93 (m, 8H, Ar—H), 7.97 (d, 1H, CH), 8.05 (d, 1H, CH), 11.99 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 56.06, 109.80, 112.25, 115.03, 118.65, 122.26, 125.87, 126.23, 129.05, 131.02, 131.48, 136.61, 164.36, 187.20. *Anal*. Calcd for $C_{18}H_{14}ClNO_2$ (311.02): C, 69.35; H, 4.53; Cl, 11.37; N 4.49%. Found: C, 68.98; H, 4.67; Cl, 11.52; N, 4.39%.

3-Chloro-2-(3-phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1H-indole (3b). A mixture of compound (**2b**) (1.4 g, 5 mmol) and hydrazine hydrate 99% (2 mL, 40 mmol) was added to ethanol, the resultant mixture was heated at reflux for 1 h, the reaction mixture was cooled, and the formed precipitate was filtered off, dried, and recrystallized from ethanol as white crystals in 90% yield, mp 256–258°C. IR (KBr): cm^{-1} 3062 (CH aromatic), 3332 (NH). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.90–7.62 (m, 9H, Ar—H), 2.66 (dd, 1H, Ha), 2.92 (dd, 1H, Hb), 5.2 (t, 1H, Hc), 11.75 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 33.98, 61.01, 105.28, 112.44, 119.52, 121.30, 124.46, 124.60, 125.39, 128.04, 128.45, 129.80, 132.68, 136.43, 150.53. MS *m/z* = (M^+−52, 243.05), (M^+, 295.05), 296.02 (M^++1), 297.08 (M^++2). *Anal*. Calcd for $C_{17}H_{14}ClN_3$ (295.05): C, 69.04; H, 4.77; Cl, 11.99; N 14.21%. Found: C, 69.32.13; H, 4.21; Cl, 12.22; N, 4.25%.

3-Chloro-2-(3-(4-methoxy)phenyl-4,5-dihydro-1H-pyrazol-5-yl)-1H-indole (3d). A mixture of compound (**2d**) (1.4 g, 5 mmol) and hydrazine hydrate 99% (2 mL, 40 mmol) in ethanol (20 mL) was refluxed for 1 h, the reaction mixture was cooled, and the formed precipitate was filtered off, washed, and recrystallized from ethanol to give the product as yellow crystal in 90% yield, mp 256–258°C. IR (KBr): cm^{-1} 2917 (CH aliphatic), 3057, 3170 (CH aromatic), 3330 (NH). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 6.90–7.62 (m, 8H, Ar—H), 6.94 (s, 1H, NH), 3.02 (dd, 1H, Ha), 3.49 (dd, 1H, Hb), 5.08 (t, 1H, Hc), 3.84 (s, 1H, CH_3), 11.75 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 40.07, 44.86, 55.69, 101.75, 112.96, 115.40, 117.84, 120.19, 122.98, 125.43, 128.22, 135.09, 160.31. *Anal*. Calcd for $C_{18}H_{16}ClN_3O$ (295.05): C, 66.36; H, 4.95; Cl, 10.88; N, 12.90%. Found: C, 66.32; H, 4.21; Cl, 10.95; N, 12.55%.

3-Chloro-2-(1,3-diphenyl-4,5-dihydro-1H-pyrazol-5-yl)-1H-indole (4b). A mixture of compound (**2b**) (1 g, 3.5 mmol) and phenylhydrazine (1 mL, 10 mmol) was added to ethanol, the resultant mixture was heated at reflux for 2 h, the reaction mixture was cooled, and the formed precipitate was filtered off, washed, and recrystallized from ethanol as white crystals in 92% yield, mp 223–225°C. IR (KBr): cm^{-1} 3053 (CH aromatic), 3324, 3398 (NH). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.11–7.45 (m, 14H, Ar—H), 2.80 (dd, 1H, Ha), 2.99 (dd, 1H, Hb), 5.42 (t, 1H, Hc), 11.10 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 34.31, 60.46, 104.30, 122.44, 117.63, 119.52, 121.30, 124.22, 124.46, 124.60, 125.81, 128.56, 128.64, 129.23, 132.76, 133.25, 136.43, 142.20, 155.43. *Anal*. Calcd for $C_{23}H_{18}ClN_3$ (371.12): C, 74.69; H, 4.36; Cl, 9.59; N, 11.36%. Found: C, 74.35; H, 4.43; Cl, 9.63; N, 11.40%.

General procedure for synthesis of compounds (5a–d). A solution of compound (**2a–d**) (3.5 mmol), thiosemicarbazide (0.31 g, 3.5 mmol), and acetic acid (2 mL) was added to ethanol, the resultant mixture was heated at reflux for 2 h, and the solid product formed after cooling the mixture was filtered and recrystallized from proper solvent.

5-(3-Chloro-1H-indol-2-yl)-3-methyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (5a). The product obtained was recrystallized from ethanol as pale yellow crystals in 80.6% yield, mp 225–227°C. IR: υ (cm^{-1}) 1236 (C═S), 3050 (CH aromatic), 3177, 3264, 3370 (NH, NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 2.32 (dd, 1H, Ha), 2.89 (dd, 2H, Hb), 2.06 (s, 3H, CH_3), 7.11 (t, 1H, Hc), 7.11–7.43 (m, 4H, Ar—H), 11.57 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 16.63, 38.06, 56.21, 103.99, 112.44, 119.52, 121.30, 124.46, 124.60, 132.98, 136.43, 155.04, 177.81. *Anal*. Calcd for $C_{13}H_{13}ClN_4S$ (292.79): C, 53.33; H, 4.48; Cl, 12.11; N, 19.14; S, 10.95%. Found: C, 53.21; H, 4.38; Cl, 12.15; N, 19.15; S, 11.14%.

5-(3-Chloro-1H-indol-2-yl)-3-phenyl-4,5-dihydro-1H-pyrazole-1-carbothioamide (5b). The product obtained was recrystallized from ethanol to give pale yellow in 88% yield, mp > 300°C. IR (KBr): cm^{-1} 3050 (CH aromatic), 3177, 3264, 3371 (NH, NH_2). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 2.98 (dd, 1H, Ha), 3.07 (dd, 1H, Hb), 3.48 (t, 1H, Hc), 6.79 (s, 2H, NH_2), 7.35–7.89 (m, 9H, Ar—H), 11.50 (s, 1H, NH). MS *m/z* (M^+-$CSNH_2$, 295.04 base peak), (M^+, 354.09). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 38.85, 57.87, 103.99, 112.44, 119.52, 121.30, 124.46, 124.60, 125.81, 125.81,128.56, 128.64, 132.76, 132.98, 136.43, 155.00, 177.81. *Anal*. Calcd for $C_{18}H_{15}ClN_4S$ (354.07): C, 60.93; H, 4.26; Cl, 9.99; N, 15.79; S, 9.03%. Found: C, 60.01; H, 4.82; Cl, 10.12; N, 15.79; S, 9.03%.

5-(3-Chloro-1H-indol-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (5d). The product obtained was recrystallized from ethanol as yellow crystal

in 88% yield, mp > 300°C. IR (KBr): cm^{-1} 3050 (CH aromatic), 3179, 3264, 3370 (NH, NH_2). ^1H NMR (400 MHz, DMSO-d_6) δ: 7.35–7.89 (m, 9H, Ar—H), 2.98 (dd, 1H, Ha), 3.07 (dd, 1H, Hb), 4.46 (t, 1H, Hc), 8.60 (s, 2H, NH_2), 11.5 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 39.80, 45.22, 110.61, 112.60, 118.65, 120.45, 125.42, 125.89, 129.49, 129.86, 136.36, 138.70, 181.96, 188.01. *Anal*. Calcd for $C_{18}H_{15}ClN_4S$ (354.07): C, 55.54; H, 3.62; Cl, 18.21; N, 14.39; S, 8.24%. Found: C, 55.65; H, 3.50; Cl, 18.29; N, 15.01; S, 8.18%.

5-(3-Chloro-1H-indol-2-yl)-3-phenyl-4,5-dihydroisoxazole (6b). A mixture of compound (**2b**) (1 g, 3.5 mmol) and hydroxylamine hydrochloride (0.33 g, 5 mmol) in ethanol (20 mL) was refluxed for 6 h, the reaction mixture was cooled, diluted with water, and neutralized with HCl, and the product formed was filtered off, dried, and recrystallized from ethanol as a white crystal in 92% yield, mp 223–225°C. IR (KBr): cm^{-1} 3057 (CH aromatic), 3253 (NH). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.11–7.45 (m, 14H, Ar—H), 2.13 (dd, 1H, Ha), 2.85 (dd, 1H, Hb), 6.25 (t, 1H, Hc), 11.75 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 34.56 (C12), 79.11 (C8), 106.84 (C7), 112.44 (C4), 119.52 (C1), 121.30 (C2), 124.46 (C3), 124.60 (C1′), 127.02 (Ph-C), 128.35 (Ph-C), 130.14 (Ph-C), 130.34 (Ph-C), 136.43 (Ph-C), 158.77 (C4′), 177.53(C6). *Anal*. Calcd for $C_{17}H_{13}ClN_2O$ (296.75): C, 68.81; H, 4.42; Cl, 11.95; N, 9.44%. Found: C, 68.50; H, 4.43; Cl, 12.02; N, 9.52%.

2-(5-(3-Chloro-1H-indol-2-yl)-3-methyl-4,5-dihydro-1H-pyrazol-1-yl)thiazol-4(5H)-one (7). A mixture of compound (**5a**) (0.5gm, 1.72 mmol), ethyl chloroacetate (0.244gm, 2 mmol), and fused sodium acetate (0.172gm, 2 mmol) were added to ethanol, the resultant mixture was heated at reflux, and then, pyridine was added till the start material disappeared; the reflux continued for 2 h. The solid product formed on hot was filtered, dried, and recrystallized from ethanol/dioxane as a yellow crystal in 81% yield, mp > 300°C. IR (KBr): cm^{-1} 2917 (CH aliphatic), 3050 (CH aromatic), 1719 (C═O), 3188 (NH). ^1H NMR (300 MHz, DMSO-d_6) δ: 2.56 (d, 1H, Ha), 2.14 (s, 3H, CH_3), 2.95 (s, 1H, Hb), 6.29 (t, 1H, Hc), 7.19–8.06 (m, 4H, Ar—H), 12.01 (s, 1H, NH Indole). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 16.63, 33.80, 38.08, 59.29, 103.99, 112.44, 119.52, 121.30, 124.46, 124.60, 132.98, 136.43, 153.82, 170.81, 182.70. *Anal*. Calcd for $C_{15}H_{13}ClN_4OS$ (332.81): C, 54.14; H, 3.94; Cl, 10.65; N, 16.84; S, 9.63%. Found: C, 54.58; H, 3.30; Cl, 10.85; N, 16.85; S, 9.60%.

4-(3-Chloro-1H-indol-2-yl)-6-phenylpyrimidine-2(1H)-thione (8). A mixture of compound (**2b**) (1 g, 3.5 mmol), thiourea (0.3 g, 4 mmol), and potassium hydroxide (1 g, 17.5 mmol) was added to ethanol, the resultant mixture was heated at reflux on water bath for 7 h, and then left overnight and neutralized with conc. HCl to give yellow precipitate, which filtered off, dried, and recrystallized from ethanol as yellow crystals in 85% yield, mp 299–300°C. IR (KBr): cm^{-1} 3081 (CH aromatic), 3138 (NH), 1232(C═S). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.28–7.86 (m, 9H, Ar—H), 8.12 (s, 1H, CH pyrimidine), 11.30 (s, 1H, NH), 12.26 (s, 1H, NH indole). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 109.41 (C9), 112.90 (C4), 117.25 (C7), 119.81 (C1), 121.47 (121.47), 124.51 (C1′), 126.03 (Ph-C), 127.14 (Ph-C), 128.83 (Ph-C), 129.23 (Ph-C), 135.71 (C5), 136.46 (Ph-C), 136.85 (C4′), 153.20 (C8), 156.38 (C12), 183.24 (C10). *Anal*. Calcd for $C_{18}H_{12}ClN_3S$ (337.15): C, 64.00; H, 3.58; Cl, 10.49; N, 12.44; S, 9.49%. Found: C, 63.84; H, 3.64; Cl, 10.52; N, 12.30; S, 9.53%.

4-(3-Chloro-1H-indol-2-yl)-6-phenylpyrimidine-2-one (9). A mixture of compound (**2b**) (1 g, 3.5 mmol), urea (0.3, 5 mmol), and potassium hydroxide (1.0 g, 17.5 mmol) was added to ethanol, and the resultant mixture was heated at reflux on a water bath for 7 h. The reaction mixture was left overnight and neutralized with conc. HCl to give yellow precipitate, which recrystallized from ethanol as a yellow crystal in 60% yield, mp 301–303°C. IR (KBr): cm^{-1} 2952(CH aliphatic), 3406 (OH, NH). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.29–8.21 (m, 9H, Ar—H), 7.95 (s, 1H, CH pyrimidine), 11.44 (s, 1H, OH), 12.87 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 104.05, 117.25, 119.81, 126.03, 127.14, 128.83, 129.23, 135.71, 136.46, 153.23, 156.80, 158.54. *Anal*. Calcd for $C_{18}H_{12}ClN_3O$ (321.15): C, 67.19; H, 3.76; Cl, 11.02; N, 13.06%. Found: C, 68.97; H, 3.80; Cl, 11.12; N, 13.10%.

4-(3-Chloro-1H-indol-2-yl)-2-oxo-6-phenyl-1,2-dihydropyridine-3-carbonitrile (10). An ethanolic mixture of compound (**2b**) (1 g, 3.5 mmol) and ethyl cyanoacetate (0.5 mL, 3.5 mmol) in the presence of ammonium acetate (0.75 g, 10 mmol) and acetic acid (5 mL) was heated at reflux for 7 h, and the solid obtained after cooling was filtered, dried, and recrystallized from dioxane/ethanol as a pale yellow crystals in 94% yield, mp 298–300°C. IR (KBr): cm^{-1} 3058 (CH aromatic), 2922 (CH aliphatic), 1678 (C═O), 2214 (CN), 3350 (NH). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 7.21–8.18 (m, 10H, Ar—H + CH Pyrimidine), 6.56 (s, 1H, NH amidic), 11.53 (s, 1H, NH indole). ^{13}C NMR (100 MHz, DMSO-d_6) (ppm) 102.98, 103.19, 111.21, 115.48, 119.41, 122.18, 125.25, 125.88, 127.14, 129.23, 129.86, 136.46, 137.51, 138.16, 142.93, 163.61. *Anal*. Calcd for $C_{20}H_{14}ClN_3O$ (347.08): C, 69.07; H, 4.06; Cl, 10.19; N, 12.08%. Found: C, 69.23; H, 3.59; Cl, 10.32; N, 12.10%.

2-Amino-4-(3-chloro-1H-indol-2-yl)-6-phenylnicotinonitrile (11). An ethanolic mixture of compound (**2b**) (1 g, 3.5 mmol) (10 mL) and malononitrile (0.23 g, 3.5 mmol) in the presence of ammonium acetate (0.75 g, 10 mmol)

and acetic acid (5 mL) was heated at reflux for 7 h, and the solid obtained after cooling was filtered, dried, and recrystallized from dioxane/ethanol as pale yellow crystals in 86% yield, mp 321–322°C. IR (KBr): cm^{-1} 2922 (CH aliphatic), 3050 (CH aromatic), 3205, 3315 (NH, NH_2), 2209(CN). ^1H NMR (300 MHz, DMSO-d_6) δ (ppm): 4.52 (s, 2H, NH_2), 7.35–8.24 (m, 10H, Ar─H + CH Pyrimidine), 11.63 (s, 1H, NH). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 86.61, 113.36, 112.75, 112.90, 115.89, 119.81, 121.47, 126.03, 128.10, 129.03, 130.45, 138.06, 141.64, 155.11, 157.84. *Anal*. Calcd for $C_{20}H_{15}ClN_4$ (346.10): C, 69.26; H, 4.36; Cl, 10.22; N, 16.15%. Found: C, 69.35; H, 3.63; Cl, 10.31; N, 16.20%.

Pharmacology. ***Antimicrobial activities.*** Some of the new synthesized compounds listed in Table 1 were screened for their *in vitro* antimicrobial activity against model Gram-positive (*S. aureus*, *Streptococcus pneumonia*, *B. cereus*, and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas sp*, *H. influenza*, *A. niger*, and *Klebsiella pneumoniae*), including multidrug-resistant species, yeasts, and mold. The Minimum Inhibition Concentrations (MICs) are compared with the results obtained for standard antibacterial ciprofloxacin, levofloxacin, moxifloxacin, clindamycin, streptomycin, gentamycin, clindamycin, and gemifloxacin and their antifungal activity against six fungal strains (*C. albicans*, *Trichophyton rubrum*, *Aspergillus flavus*, *Fusarium oxysporum*, *Scopulariopsis brevicaulis*, and *G. candidum*). The antimicrobial activities of the tested compounds were evaluated by the reported method [16] using 0.005% (50 μg/mL) concentration of selected compounds in DMSO as a solvent. The inhibition zone (mm) was compared with clotrimazole as standard for antifungal. In the case of antibacterial activity, the inhibition zone (mm) was compared with a series of antibiotics according to the sensitivity of each type of bacteria to the most effective antibiotic for it as a standard. The biological activity as expressed by the growth inhibition zone of the tested microorganism was listed in Tables 1 and 2.

Anti-inflammatory activity. Anti-inflammatory activity was measured using carrageenan-induced rat paw edema assay [17]. Edema was induced by subplantar injection adult albino rats, weighing 150–200 g, were used. The animals were allowed food and water *ad libitum*, except during the experiment. They were housed in a room at 23 ± 2°C with a 12-h light/dark cycle. The animals were randomly allocated into groups of six animals each at the beginning of the experiment and were fasted for 24 h before the experiment with free access to water. All of the compounds and the reference drug were suspended in a 0.5% carboxymethyl cellulose solution. The standard drug indomethacin was administered orally at a dose of 20 μmol/kg^{-1}. The tested compounds were administered orally at an equimolar oral dose relative to 20 μmol/kg^{-1} of indomethacin. The control group received a 0.5% carboxymethyl cellulose solution. Into the subplantar region of the right hind paw of each rat, 0.1 mL of 1% carrageenan solution in saline was injected subcutaneously, 1 h after the administration of the test compounds and standard drug. The right paw volume was measured using a digital plethysmometer (Model 7150, Ugo Basile, Varese, Italy), directly before and after 1-, 2-, 3-, and 4-h intervals after administration of the tested compounds. The percent edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation.

Statistical analysis. The results were analyzed by one-way analysis of variance followed by Newman–Keuls multiple comparison test as a post-test. These analyses were carried out using computer PRISM program for windows, version 3.0 (Graph Pad software, Inc., San Diego, CA). The significance difference between groups was accepted at $P < 0.05$*, 0.01**, or 0.001***, and the data were expressed as mean ± standard error.

The inflammatory response is represented as the time course of the percentage of the increase in paw swelling as the area under the curve response and as the percent edema inhibition was calculated from the mean effect in the control and treated animals according to the following equation:

$$\text{Percent edema inhibition} = (1 - V_t/V_c) \times 100,$$

where V_t represents the mean increase in paw volume in rats treated with tested compounds and V_c represents the mean increase in paw volume in the control group of rats.

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SUPPORTING INFORMATION

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