Volume2, Issue1, January 2011
Available Online at www.ijppronline.com
International Journal Of Pharma Professional's Research

Research Article

SYNTHESIS AND ANTI-INFLAMMATORY-ANTIMICROBIAL ACTIVITY OF INDOLIN-2-ONE DERIVATIVES

Mohammad Shaquiquzzaman[1*], Suroor Ahmad Khan[1], Mohammad Amir[1], Mohammad Shahar Yar[1], Mohammad Mumtaz Alam[1]

ISSN NO:0976-6723

1) Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi-110 062, India

Abstract

A series of 1-phenyl-3-(2-{substituted-phenylhydrazono})-indolin-2-one derivatives (**IIa-k**) were synthesized. The compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and antimicrobial actions. Among the tested compounds, two compounds **IIf** and **IIk** showed higher degree of anti-inflammatory activity (>75% activity of standard indomethacin). These compounds were further tested for analgesic activity in acetic acid induced writhing test and showed interesting protection comparable to that of standard. The compounds were also tested for their ulcerogenic action and showed superior GI safety profile. Compound **IIk** was found to have additional antimicrobial activity with MIC 12.5µg/mL against *S.aureus* and *E.coli*.

Keywords: - Indolin-2-one; anti-inflammatory; analgesic; antimicrobial activity.

Introduction

Substituted indolin-2-ones are an important class of biologically active molecules. Pharmacological activity reported in literature include the anti-inflammatory [1-2], analgesic [1], COX-2 inhibition [3-4], antifungal [5-6], antibacterial [7-8], anticonvulsant [9-10], antioxidant [11], antiviral [12] etc.

Owing to the versatility of indolin-2-one we have synthesized eleven new 1-phenyl-3-(2-{substituted-phenylhydrazono})-indolin-2-one derivatives by condensing different benzene diazonium chloride with *N*-phenyl indoline-2-one and evaluated for their anti-inflammatory and analgesic activity. They were also tested for their gastro-intestinal damage by measuring their severity index (SI). The synthesized derivatives were also tested for antibacterial activity. We also report the effect of such molecular variations on the anti-inflammatory and antimicrobial activities of indolin-2-one nucleus.

Material And Method

Chemistry

Chemicals were purchased from Merck and Sigma-Aldrich as 'synthesis grade' and used without further purification. Melting points (m.p.) were determined in open capillary tubes and are uncorrected. Elemental analyses were performed on a Perkin-Elmer analyzer and were in range of ± 0.4% for each element analyzed (C, H, N). The IR spectra were measured as potassium bromide pellets using a Perkin-Elmer 1725X spectrophotometer. ^{1}H-NMR spectra were recorded on Bruker Avance-400 MHz in $CDCl_3$ or DMSO with tetramethylsilane (TMS) as an internal standard; chemical shifts (δ) are reported in parts per million (*ppm*) downfield from TMS. Mass spectroscopic analyses for compounds were performed on a JEOL JMS-D 300 instrument fitted with a JMS 2000 data system at 70eV. Spectral data are consistent with assigned structures. Thin-layer chromatography was carried out to monitor the reactions using silica gel (Merck No. 5554). Dry solvents were used throughout.

*General procedure for synthesis of Substituted Benzene diazonium chloride (**Ia-k**)*

The synthesis of compounds (**Ia-k**) were carried out by literature procedure [13].

*General procedure for the synthesis of 1-phenyl-3-(2-{substituted-phenylhydrazono})-indoline -2-one derivatives (**IIa-k**)*

Substituted benzene diazonium salt solution (**Ia-k**) (1mmole) were added dropwise with continuous stirring to a ice-cold mixture of *N*-phenyl indoline-2-one (1mmole) and sodium acetate (5mmole) in pyridine. The stirring was continued for 30minutes and the reaction mixture was then left overnight at room temperature. The solid separated was collected and recrystallized with glacial acetic acid.

1 Phenyl-3-(2-phenylhydrazono)-indoline -2-one - (IIa). Yield: 85%, m.p. 225-26°C. ^{1}H-NMR ($CDCl_3$) δ 6.78-7.76 (m, 14H, ArH), 12.32 (bs, 1H, NH); IR (cm^{-1}, KBr): 3210, 1676, 1590; MS: *m/z* 312(M^+), Anal. Calcd. for

Correspondence Address:
M. Shaquiquzzaman*
Asstt. Professor, Department of Pharmaceutical chemistry,
Faculty of Pharmacy, Jamia Hamdard (Hamdard University),
New Delhi-110 062, India
PH: +91-11-26059681, 26059688, Mobile- +91-9990663405
E-mail: shaqiq@gmail

$C_{20}H_{15}N_3O$: C, 76.66; H, 4.82; N, 13.41. Found: C, 76.48; H, 4.81; N, 13.40.

1-Phenyl-3-(2-{2-methyl-phenylhydrazono})-indoline -2-one (IIb). Yield: 65%, m.p. 261-62°C. ^{1}H-NMR ($CDCl_3$) δ 2.33 (s, 3H, CH_3), 6.73-7.71 (m, 13H, ArH), 12.89 (bs, 1H, NH); IR (cm^{-1}, KBr): 3215, 1680, 1588; MS: *m/z* 326(M^+), Anal. Calcd. for $C_{21}H_{17}N_3O$: C, 77.04; H, 5.23; N, 12.84. Found: C, 77.28; H, 5.24; N, 12.83.

1-Phenyl-3-(2-{4-methyl-phenylhydrazono})-indoline -2-one (IIc). Yield: 63%, m.p. 255-56°C. ^{1}H-NMR ($CDCl_3$) δ 2.39 (s, 3H, CH_3), 6.68-7.78 (m, 13H, ArH), 12.36 (bs, 1H, NH); IR (cm^{-1}, KBr): 3220, 1668, 1588; MS: *m/z* 326(M^+), Anal. Calcd. for $C_{21}H_{17}N_3O$: C, 77.04; H, 5.23; N, 12.84. Found: C, 77.32; H, 5.24; N, 12.84..

1-Phenyl-3-(2-{2-methoxy-phenylhydrazono})-indoline -2-one (IId). Yield: 58%, m.p. 245-46°C. ^{1}H-NMR ($CDCl_3$) δ 3.81 (s, 3H, OCH_3), 6.63-7.81 (m, 13H, ArH), 12.42 (bs, 1H, NH); IR (cm^{-1}, KBr): 3178, 1665, 1588; MS: *m/z* 342(M^+), Anal. Calcd. for $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.68; H, 4.98; N, 12.23..

1-Phenyl-3-(2-{4-methoxy-phenylhydrazono})-indoline -2-one (IIe). Yield: 58%, m.p. 237-38°C. ^{1}H-NMR ($CDCl_3$) δ 3.79 (s, 3H, OCH_3), 6.69-7.76 (m, 13H, ArH), 12.49 (bs, 1H, NH); IR (cm^{-1}, KBr): 3181, 1673, 1599; MS: *m/z* 342(M^+), Anal. Calcd. for $C_{21}H_{17}N_3O_2$: C, 73.45; H, 4.99; N, 12.24. Found: C, 73.72; H, 4.94; N, 12.23..

1-Phenyl-3-(2-{2-chloro-phenylhydrazono})-indoline -2-one (IIf). Yield: 43%, m.p. 259-60°C. ^{1}H-NMR ($CDCl_3$) δ 6.73-7.86 (m, 13H, ArH), 12.16 (bs, 1H, NH); IR (cm^{-1}, KBr): 3211, 1676, 1610; MS: *m/z* 347(M^+), Anal. Calcd. for $C_{20}H_{14}ClN_3O$: C, 69.07; H, 4.06; N, 12.08. Found: C, 69.28; H, 4.05.; N, 12.07.

1-Phenyl-3-(2-{3-chloro-phenylhydrazono})-indoline -2-one (IIg). Yield: 53%, m.p. 253-54°C. ^{1}H-NMR ($CDCl_3$) δ 6.70-7.82 (m, 13H, ArH), 12.30 (bs, 1H, NH); IR (cm^{-1}, KBr): 3220, 1672, 1608; MS: *m/z* 347(M^+), Anal. Calcd. for $C_{20}H_{14}ClN_3O$: C, 69.07; H, 4.06; N, 12.08. Found: C, 69.42; H, 4.07; N, 12.07.

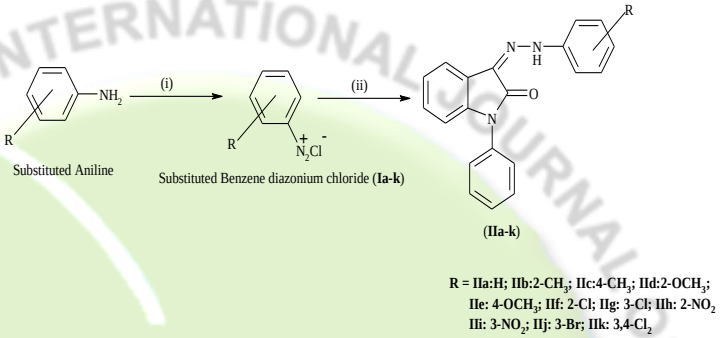
1-Phenyl-3-(2-{2-nitro-phenylhydrazono})-indoline -2-one (IIh). Yield: 36%, m.p. 256-58°C. ^{1}H-NMR ($CDCl_3$) δ 6.76-8.13 (m, 13H, ArH), 12.73 (bs, 1H, NH); IR (cm^{-1}, KBr): 3216, 1681, 1603; MS: *m/z* 357(M^+), Anal. Calcd. for $C_{20}H_{14}N_4O_3$: C, 67.03; H, 3.94; N, 15.63. Found: C, 67.31; H, 3.93; N, 15.64.

1-Phenyl-3-(2-{3-nitro-phenylhydrazono})-indoline -2-one (IIi). Yield: 44%, m.p. 249-50°C. ^{1}H-NMR ($CDCl_3$) δ 6.80-8.23 (m, 13H, ArH), 12.65 (bs, 1H, NH); IR (cm^{-1}, KBr): 3241, 1678, 1612; MS: *m/z* 357(M^+), Anal. Calcd. for $C_{20}H_{14}N_4O_3$: C, 67.03; H, 3.94; N, 15.63. Found: C, 67.27; H, 3.94; N, 15.65.

1-Phenyl-3-(2-{3-bromo-phenylhydrazono})-indoline -2-one (IIj). Yield: 58%, m.p. 267-68°C. ^{1}H-NMR ($CDCl_3$) δ 6.78-7.86 (m, 13H, ArH), 12.71 (bs, 1H, NH); IR (cm^{-1}, KBr): 3208, 1668, 1606; MS: *m/z* 391(M^+), Anal. Calcd. for $C_{20}H_{14}NBrN_3O$: C, 61.24; H, 3.60; N, 10.71. Found: C, 61.42; H, 3.59; N, 10.70.

*1-Phenyl-3-(2-{3,4-dichloro-phenylhydrazono})-indoline -2-one (**IIk**)*. Yield: 56%, m.p. 285-86°C. ^{1}H-NMR ($CDCl_3$) δ 6.75-7.90 (m, 12H, ArH), 12.78 (bs, 1H, NH); IR (cm^{-1}, KBr): 3230, 1673, 1608; MS: *m/z* 381(M^+), Anal. Calcd. for $C_{20}H_{13}Cl_2N_3O$: C, 62.84; H, 3.43; N, 10.99. Found: C, 62.36; H, 3.44; N, 10.98.

SCHEME-I:



Reagents and condition: (i) Sodium nitrite, conc. HCl, 0-5°C; (ii) N-Phenylindolin-2-one, Sodium acetate, pyridine, stirring

Biological Activity

Anti-inflammatory activity: The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan-induced paw edema method of Winter *et al* [14]. The experiment was performed on Albino rats of Wistar strain of either sex, weighing 180-200 g. The animals were randomly divided into groups of six. Group I was kept as control, and received only 0.5% carboxymethyl cellulose (CMC) solution. Groups II was kept as standard and received Indomethacin (10mg/kg *p.o.*). Carrageenan solution (0.1% in sterile 0.9% NaCl solution) in a volume of 0.1mL was injected subcutaneously into the sub-plantar region of the right hind paw of each rat, 30min after the administration of the

test compounds and standard drugs. The paw volume was measured by saline displacement shown on screen of digital Plethysmometer (Ugo Basile) at 2 and 3 hrs after carrageenan injection. Thus the edema volume in control group (V_c) and edema volume in groups treated with test compounds (V_t) was measured and the percentage inhibition of edema was calculated using the formula:

Anti-inflammatory activity (% inhibition) = $(V_c - V_t)/ V_c \times 100$

Analgesic activity: Compounds which showed anti-inflammatory activity above 75% of Indomethacin were screened for analgesic activity. Analgesic activity was done by acetic acid induce writhing method [15].

Swiss albino mice (25–30 g) of either sex were divided into group of six in each. A 1% aqueous acetic acid solution

(i.p. injection in a volume of 0.1 mL) was used as writhing induced agent. Mice were kept individually in the test cage, before acetic acid injection and habituated for 30min. Screening of analgesic activity was performed after *p.o.* administration of test drugs at a dose of 10mg/kg. Group I was taken as control and received CMC suspension only, group II received reference drug Indomethacin and rest of the groups were treated with test drugs (10mg kg^{-1}) suspended in 1.0% CMC orally. After 1h of drug administration 0.10mL of 1% acetic acid solution was given to mice intraperitoneally. Stretching movements consisting of arching of the back, elongation of body and extension of hind limbs were counted for 5–15 min of acetic acid injection. The analgesic activity was expressed in terms of percentage inhibition.

$$\%\text{Analgesic activity} = \{(n - n')/ n\} \times 100$$

where, n = mean number of writhes of control group, n' = mean number of writhes of test group.

Acute ulcerogenesis: Acute ulcerogenesis test was done according to Cioli *et al*. [16]. Albino rats (150–200 g) were divided into different groups consisting of six animals in each group. Ulcerogenic activity evaluated after *p.o.* administration of test compounds or Indomethacin at the dose of 30mg/kg. Control rats received *p.o.* administration of vehicle (suspension of 1% methyl cellulose). Food but not water was removed 24 h before administration of the test compounds. After the drug treatment the rats were fed with normal diet for 17 h and then sacrificed. The stomach was removed and opened along the greater curvature, washed with distilled water and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring system: 0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streaks, 2.0: ulcers > 3 but < 5, 3.0: ulcers > 5. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Antibacterial activity: The newly prepared compounds were screened for their antibacterial activity against *Escherichia coli* (ATCC-8739) and *Staphylococcus aureus* (ATCC-29737) bacterial strains at a concentration of 100 μg/ml by turbidity method [17] using norfloxacin as standard. Compounds inhibiting growth of one or more of the above microorganisms were further tested for minimum inhibitory concentration (MIC).

RESULT AND DISCUSSION

Chemistry

The title compounds 1-phenyl-3-(2-{substituted-phenylhydrazono})-indolin-2-one derivatives (**IIa-k**) was synthesized by using Scheme-1. The substituted benzene diazonium chlorides (**Ia-k**) were synthesized by following the previously reported methods in high yield [13]. The obtained benzene diazonium chlorides (**Ia-k**) were condensed with *N*-phenyl indolin-2-one in presence of sodium acetate in pyridine to give 1-phenyl-3-(2-{substituted-phenylhydrazono})-indolin-2-one derivatives (**IIa-k**).

In the IR spectral data all the compounds showed two peaks each around 3210cm^{-1} and 1680cm^{-1} indicating the presence of NH and carbonyl group respectively. ^{1}H-NMR spectral data of all the compounds showed characteristic peak at appropriate δ-values. The synthesized compounds also gave M^+ peak in reasonable intensities.

Biological screening

Anti-inflammatory activity:

Compounds **IIa**-**IIk** were tested *in-vivo* for their anti-inflammatory activity by carrageenan-induced rat paw edema method [14]. Indomethacin was used as a standard drug for comparison. The results of the pharmacological evaluation are listed in Table 1. Three of the eleven tested compounds showed statistically significant anti-inflammatory activity with respect to standard. The maximum activity was shown by the compounds having electronegative substitution i.e. 1-phenyl-3-(2-{3,4-dichloro-phenylhydrazono})-indolin-2-one derivative **IIk**, 1-phenyl-3-(2-{3-bromo-phenylhydrazono})-indolin-2-one derivative **IIj** and 1-phenyl-3-(2-{2-chloro-phenylhydrazono})-indolin-2-one derivative **IIf** with 66.26%, 46.53% and 42.65% inhibition respectively. Further, when the chloro group was replaced by methoxy group (Compound **IIe**) or methyl group (Compound **IIb**) the activity decreased.

Test compounds that exhibited good anti-inflammatory activity **IIf**, **IIj** and **IIk** were further evaluated for their analgesic, ulcerogenic and antibacterial activity.

Analgesic activity

The compounds that exhibited above 75% of anti-inflammatory activity of Indomethacin were evaluated for analgesic effects using acetic acid induced writhing method [15]. The results of analgesic activity (Table-1) indicated that compounds **IIj** and **IIk** showed 49.62% and 46.53% protection against acetic acid induced writhings. Compound **IIf** also showed good analgesic activity.

According to structure activity relationship, it is clear that the dichloro substituted indolin-2-one derivatives was found to be a good anti-inflammatory agent with analgesic activity.

Acute Ulcerogenesis

The compounds which were screened for analgesic activity were further tested for their acute ulcerogenic activity. Compounds **IIf**, **IIj** and **IIk** were tested according to the method reported by Cioli *et al* [16]. The tested compounds showed low ulcerogenic activity ranging from 0.17±0.11 to 0.34±0.17 whereas the standard drug Indomethacin showed

high severity index of 0.84±0.17. The maximum reduction in ulcerogenic activity (0.17±0.11) was found in the dichloro substituted and bromo substituted derivatives of indoli-2-one. Results are presented in Table-1.

Table 1: Anti-inflammatory and analgesic activity along with ulcerogenic effect of the synthesized compounds IIa-IIk.

Compound	% Inhibition ± SEM[a]		Analgesic activity (Writhing test)[a]		Severity index[b]
	After 2 hr	After 3 hr	No. of writhes/30min	% Protection	
Control	-	-	-	-	0.00±0.00
Indomethacin	67.85±1.42	76.01±0.81	16.34±0.6667	60.302±2.78	0.84±0.17**
IIa	25.92±1.19**	40.65±0.81**	-	-	-
IIb	20.47±1.02**	34.14±1.09**	-	-	-
IIc	15.71±0.82**	31.30±0.92**	-	-	-
IId	35.47±1.35**	48.57±1.19**	-	-	-
IIe	37.14±1.22**	51.42±1.23**	-	-	-
IIf	42.61±2.00**	56.70±1.53**	23.83±1.07**	42.65±2.23**	0.34±0.17
IIg	39.04±1.14**	53.65±0.44**	-	-	-
IIh	32.14±0.95**	45.93±0.60**	-	-	-
Iii	30.47±1.02**	43.90±1.17**	-	-	-
IIj	44.76±1.83**	58.13±2.05**	22.00±0.57**	46.53±3.38**	0.17±0.11
IIk	49.76±1.53**	66.26±0.92**	20.83±0.54**	49.62±2.27*	0.17±0.11

*p< 0.05; **p<0.01. [a]Relative to their respective standard (Indomethacin) and data were analyzed by one-way ANOVA followed by Dunnett's test for n = 6. [b]Relative to the control and data were analyzed by one-way ANOVA followed by Dunnett's test for n = 6.

Thus these studies showed that synthesized compounds also have less gastro-intestinal irritation or showed better gastric safety profile.

Antimicrobial activity*:*

Compounds **IIf**, **IIj** and **IIk** were also tested for their antibacterial activity by using turbidity method [17] against *Staphylococcus aureus* representing Gram-positive bacteria and *Escherichia coli* representing Gram negative bacteria The results showed that compound **IIk** was active against both strain with MIC of 12.5 µg / mL (Table-2).

Table 2: Antibacterial and antifungal study; MIC results of IIf, IIj and IIk

Compounds	*Staphylococcus aureus*	*Escherichia coli*
Norfloxacin	6.25	6.25
IIf	25	50
IIj	25	50
IIk	12.5	12.5

MIC (µg/mL)=minimum inhibitory concentration, i.e., the lowest concentration to completely inhibit bacterial growth

Conclusion

Eleven new indolin-2-one derivatives were synthesized and screened for anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities. It was interesting to note that one compound **IIk** was found to have good anti-inflammatory and analgesic activity. This compound also showed superior GI safety profile as indicated by its ulcerogenicity.

Compound **IIk** was also found to have MIC of 12.5µg/mL against both bacterial strains.

Thus the indolin-2-one derivatives were found having dual functional properties i.e. anti-inflammatory-analgesic and antibacterial and represent a promising class of compounds with an interesting pharmacological profile.

Acknowledgement:

The authors are grateful to Janab Abdul Mueed for providing infrastructure and facility to carry out this work.

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