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Research Article

SYNTHESIS, CHARACTERIZATION, AND ANTHELMINTIC ACTIVITY OF ISATIN ANALOGS AGAINST *PHERITIMA POSTHUMA*

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ABSTRACT

Objective: Isatin (1H-indole-2,3-dione), an endogenous compound identified in many organisms, shows a wide range of biological activities. In view of these facts, in our present communication, we wish to report the synthesis and evaluation of some isatin analogs for anthelmintic activity against *Pheritima posthuma*.

Methods: A series of 16 isatin analogs (3a-3p) have been synthesized, characterized by physical and spectral data (Fourier transform infrared, ^{1}H nuclear magnetic resonance and mass) and screened for anthelmintic activity against *P. posthuma* at various concentrations (5, 10, and 20 mg/ml).

Results: All compounds were tested for the beginning of paralysis time followed by a time of the death of worms. All prototypes at the concentration of 20 mg/ml showed significant activity ($p<0.01$) compared to that of control. The compounds 3f and 3i were found to possess significant activity compared to standard albendazole (20 mg/ml).

Conclusion: The study encourages us to consider a new molecular skeleton of isatin substituted at the first and third position by aryl groups with adequate spacers which may be identified as potential leads for the development of future studies in various *in vivo* models for anthelmintic activities.

Keywords: Isatin, Synthesis, Spectral analysis, *Pheritima posthuma*, Anthelmintic activity.

INTRODUCTION

Anthelmintics or anti-helminthics are drugs that force out parasitic worms (helminths) and other internal parasites from the body by either stunning or killing them and without causing significant damage to the host [1]. Moreover, the discovery of an anthelmintic vaccine [2], frequently delayed by difficulties in drug development, has made the fight against parasites a major economic and food security issue. Helminthes infections, repetitively entitled helminthiasis is among the most invasive infection and a prime degenerative disease for a large proportion of world's population. In developing countries, they pose a large threat to public health and contribute to the prevalence of malnutrition, anemia, eosinophilia, and pneumonia [3]. The helminthes mainly survive in the human body in the intestinal tract, but they are also found in tissue, as their larvae migrate toward them [4]. Most diseases caused by helminthes are of a chronic, unbearable nature; they most likely cause more morbidity and better economic and social deficiency among humans and animals than any single group of parasites. Chemical control of helminthes coupled with the better administration has been the important worm control strategy throughout the world. However, development of resistance in helminthes [5,6] against conventional anthelmintics is a leading problem in the treatment of helminthes diseases [7,8]. Despite this prevalence of parasitic infections, the search on the anthelmintic drug is sparse. Helminths have complex life-cycles, special knowledge of which is required for the treatment of the infections caused by them [9]. Therefore, it is important to look for alternative strategies against gastrointestinal nematodes, which have led to the anthelmintic activity.

Isatin (1H-indole-2, 3-dione) attracts the greatest interest among indole derivatives [10]. Erdman and Laurent in 1841 proved that isatin was obtained from the oxidation of indigo dye by nitric acid and chromic acids [11]. The attractive isatin derivatives have a vital role in synthetic drugs and biological processes medicinal chemistry involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. Isatin moiety has a wide variety of interesting biological activities such as antiviral, antioxidant, anti-proliferative, antimicrobial, and antifungal activities [12-24]. Our previous communication has reported the synthesis and evaluation of some novel isatin analogs [25] as antimicrobial agents. In the present investigation, we report novel isatin analogs as promising anthelmintics.

METHODS

All the chemicals and solvents were purchased from Sigma Aldrich and Spectrochem. Unless otherwise mentioned the solvents were used without purification. Reactions were monitored by thin-layer chromatography (TLC) on precoated silica gel plates (Kieselgel 60 F 254, Merck) and the spots were detected under ultraviolet light (254 nm). Purification was performed by column chromatography using silica gel (particle size 100-200 mesh, CDH). Melting points were determined using Optimelt (Stanford Research Systems, Sunnyvale, CA 94089) by the capillary method and were uncorrected. Infrared (IR) spectra were taken on a Fourier transform IR Spectrophotometer IR-Prestige 21 (Shimatzu Corporation, Japan) from 4000 to 400/cm using KBr discs. ^{1}H Nuclear magnetic resonance (NMR) Spectra were recorded on a Varian spectrometer Spectrophotometer 400 MHz using dimethyl sulfoxide-d6 (DMSO-d_6) as a solvent. Chemical shifts are expressed in delta values (ppm) relative to tetramethylsilane as an internal standard. The peak multiplicity is reported as s (singlet), d (doublet), t (triplet) and m (multiplet). Mass spectra were recorded with using (ESI^+) MS. Saline water (Claris Lifesciences Ltd, Ahmedabad), albendazole (pure drugs) and vehicle (2% v/v Tweens 80 in distilled water were used. All the prototypes were dissolved in minimum quantity of 2% v/v Tween 80 and then the volume was adjusted to 10 ml with normal saline for making the concentration of 5, 10 and 20 mg/ml).

General procedure for synthesis of intermediates 1, 3-dihydro-indol-2-one (1_ia-b) [25]

According to Schiff base procedure, equimolar quantities (0.004 mol) of isatin and appropriately substituted aniline (0.004 mol) were taken and dissolved in appropriate warm ethanol in the presence of 2-3 drops of

glacial acetic acid and refluxed for 2-3 hrs [26,27]. After completion of the reaction, the reaction mixture was kept overnight to get the yellow solid product. The product was filtered, dried and recrystallized from ethanol.

3-(3-chlorophenylimino) indolin-2-one (1_ia)

Yield: 84%, mp: 235-237°C.

IR (KBr, cm^{-1}): 3220.27 (enolic O-H stretching), 1747.57 (C=O stretching), 1658.84 (C=N stretching). 1464.98(C=C), 680(C-Cl)

3-(4-chlorophenylimino) indolin-2-one (1_ib)

Yield: 93%, mp: 258-260°C.

IR (KBr, cm^{-1}): 3268.19 (enolic O-H stretching), 1739.85 (C=O stretching), 1653.5 (C=N stretching). 1464.20 (C=C stretching), 680.2(C-Cl)

General procedure for preparation of 2-chloro-N-phenyl acetamide derivatives (2_ia-h) [25]

Appropriately substituted aniline (0.033 mol) was dissolved in 12.5 ml glacial acetic acid. 2-chloroacetyl chloride (2.95 ml, 0.037 mol) was added drop-wise to this solution while cooling in ice-bath. At first, the reaction mixture was stirred in ice-bath for 1 hr and then stirred for 2 hrs in room temperature. Next, the mixture was poured into saturated sodium acetate solution. The precipitate was filtered and washed with 5-7 times in cold water [28]. Crystallization from ethanol-water mixture yielded a crystalline mass.

2-chloro-N-phenylacetamide (2_ia)

Yield: 91%, mp: 145-146°C.

IR (KBr, cm^{-1}): 3267 (N-H stretching), 1670 (C=O stretching), 1350 (C-N stretching).

2-chloro-N-o-tolylacetamide (2_ib)

Yield: 89%, mp: 105-107°C.

IR (KBr, cm^{-1}): 3267 (N-H stretching), 1662 (C=O stretching), 1458 (C-CH_3 stretching), 1330 (C-N stretching).

2-chloro-N-p-tolylacetamide (2_ic)

Yield: 90%, mp: 175-177°C.

IR (KBr, cm^{-1}): 3273 (N-H stretching), 1674 (C=O stretching), 1450 (C-CH_3 stretching), 1344 (C-N stretching).

2-chloro-N-(3-methoxyphenyl) acetamide (2_id)

Yield: 87%, mp: 119-121°C.

IR (KBr, cm^{-1}): 3286 (N-H stretching), 1653 (C=O stretching), 1340 (C-N stretching), 1103 (C-OCH_3 stretching).

2-chloro-N-(4-methoxyphenyl) acetamide (2_ie)

Yield: 76%, mp: 119-120°C.

IR (KBr, cm^{-1}): 3294 (N-H stretching), 1666 (C=O stretching), 1346 (C-N stretching), 1112 (C-OCH_3 stretching).

2-chloro-N-(2,4-dimethylphenyl) acetamide (2_if)

Yield: 64%, mp: 148-149°C.

IR (KBr, cm^{-1}): 3251 (N-H stretching), 1651 (C=O stretching), 1537 (C-CH_3 stretching), 1336 (C-N stretching).

2-chloro-N-(2, 5-dimethylphenyl) acetamide (2_ig)

Yield: 68%, mp: 148-150°C.

IR (KBr, cm^{-1}): 3258 (N-H stretching), 1662 (C=O stretching), 1548 (C-CH_3 stretching), 1340(C-N stretching).

2-chloro-N-(3, 4-dimethylphenyl) acetamide (2_ih)

Yield: 75%, mp: 146-147°C.

IR (KBr, cm^{-1}): 3262 (N-H stretching), 1670(C=O stretching), 1540 (C-CH_3 stretching), 1349(C-N stretching).

General preparation of synthesis of the title compounds (3a-3p) [25]

A small amount of K_2CO_3 (15 mmol) was added to the synthesized Schiff bases of isatin (10 mmol) and the reaction mixture was stirred at room temp for 1 hr in 8-10 ml of anhydrous dimethylformamide. After completion of 1 hr, the solution turned red-brown in color. Then cholroanilides (10 mmol) and KI (2 mmol) was added to this solution and refluxed for 3-12 hrs [29,30]. In between TLC was checked to confirm the completion of reaction (Et Ac: Hxn, 40:60). After completion of the reaction, the mixture was poured into ice-cold water. The crude product was filtered and washed thoroughly with cold water in several times. The final compounds were recrystallized from ethanol-water mixture (EtOH: H_2O::1:1). The molecular formula, reaction times, yields and melting points are depicted in Table 1.

2-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-phenylacetamide (3a)

Pale yellow solid, IR (KBr, cm^{-1}): 3326 (NH stretching), 3140, 3078 (Ar-CH stretching), 1738, 1680 (C=O stretching), 1602 (C=N stretching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.659 (s, 2H, -CH_2-), 6.451 (d, 1H, J=7.6 Hz, Ar-H), 6.877 (t, 1H, J=8 Hz, 7.6 Hz, Ar-H), 7.014 (d, 1H, J=8 Hz, Ar-H), 7.065-7.164 (m, 3H, Ar-H), 7.332 (t, 3H, J=8.4 Hz, 8.4 Hz, Ar-H), 7.458 (t, 1H, J=8 Hz, 7.2 Hz, Ar-H), 7.511-7.595 (m, 3H, Ar-H), 10.374 (s, 1H, -NH-). MS (ESI) m/z=389.9 $(M+1)^+$; cald for, $C_{22}H_{16}ClN_3O_2$: 389.83.

2-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-phenylacetamide (3b)

Orange powder, IR (KBr, cm^{-1}): 3312 (N–H streching), 3174, 3064 (Ar–CH streching), 1739, 1680 (C=O streching). 1603 (C=N streching), ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 4.655 (s, 2H, -CH_2-), 6.532 (d, 1H, J=7.2 Hz, Ar-H), 6.882 (t, 1H, J=7.6, 7.6 Hz, Ar-H), 7.066-7.141 (m, 3H, Ar-H), 7.311-7.391 (m, 3H, Ar-H),7.453 (t,1H, J=7.6, 7.2 Hz, 8 Ar-H), 7.533-7.594 (m, 4H, Ar-H), 10.373 (s, 1H, -NH-). MS (ESI) m/z = 390.0 $(M+1)^+$; cald for, $C_{22}H_{16}ClN_3O_2$: 389.83.

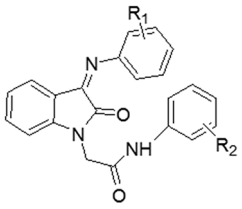
2-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-o-tolylacetamide (3c)

Yellow solid crystal, IR (KBr, cm^{-1}): 3257 (NH stretching), 3064, 2962 (Ar-CH stretching), 1734, 1700 (C=O stretching), 1602 (C=N stretching) ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.209 (s, 3H, -CH_3-), 4.676 (s, 2H, -CH_2-), 6.429 (d, 1H, J=7.6 Hz, Ar-H), 6.801-6.839 (t, 1H, J=7.6, 7.6 Ar-H), 7.01 (d, 1H, J=7.2 Hz, Ar-H), 7.092-7.189 (m, 3H, Ar-H), 7.228-7.276 (d, 1H, J=6.8Hz, Ar-H), 7.265-7.372 (m, 2H, Ar-H), 7.265-7.372 (m, 3H, Ar-H), 9.729 (s, 1H, -NH-). MS (ESI) m/z=404.0 $(M+1)^+$; cald for, $C_{23}H_{18}ClN_3O_2$: 403.86.

2-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-p-tolylacetamide (3d)

Brownish yellow solid, IR (KBr, cm^{-1}): 3312 (N–H stretching), 3198, 2936 (Ar–CH stretching), 1733, 1696 (C=O stretching), 1601 (C=N stretching), ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.256 (s, 3H, -CH_3-),4.634 (s, 2H, -CH_2-), 6.448 (d, 1H, J=8 Hz, Ar-H), 6.875 (t, 1H, J=7.6, 8 Hz Ar-H), 7.013 (d, 1H, J=8 Hz, Ar-H), 7.101-7.190 (m, 4H, Ar-H), 7.346 (d, 1H, J=8 Hz, Ar-H), 7.413-7.550 (m, 4H, Ar-H), 10.278 (s,

Table 1: Reaction time, melting point, yield and molecular formula of the title compounds (3a-3p)



(3a-3p)

Compound	R_1	R_2	Reaction time (hrs)[a]	M.p(°C)	Yield	Molecular formula
3a	-3Cl	-H	6	220-222	75	$C_{22}H_{16}ClN_3O_2$
3b	-4Cl	-H	8	214-216	74	$C_{22}H_{16}ClN_3O_2$
3c	-3Cl	-2-CH_3	9	216-218	61	$C_{23}H_{18}ClN_3O_2$
3d	-3Cl	-4-CH_3	4	194-196	68	$C_{23}H_{18}ClN_3O_2$
3e	-4Cl	-2-CH_3	7	259-261	67	$C_{23}H_{18}ClN_3O_2$
3f	-4Cl	-4-CH_3	4	180-182	68	$C_{23}H_{18}ClN_3O_2$
3g	-3Cl	-3-OCH_3	3	154-156	74	$C_{23}H_{18}ClN_3O_3$
3h	-3Cl	-4-OCH_3	8	214-215	71	$C_{23}H_{18}ClN_3O_3$
3i	-4Cl	-3-OCH_3	5.5	226-228	73	$C_{23}H_{18}ClN_3O_3$
3j	-4Cl	-4-OCH_3	6	249-251	71	$C_{23}H_{18}ClN_3O_3$
3k	-3Cl	-2,4-CH_3	7	229-231	65	$C_{24}H_{20}ClN_3O_2$
3l	-3Cl	-2,5-CH_3	6	232-233	69	$C_{24}H_{20}ClN_3O_2$
3m	-3Cl	-3,4-CH_3	12	219-221	70	$C_{24}H_{20}ClN_3O_2$
3n	-4Cl	-2,4-CH_3	7	262-263	74	$C_{24}H_{20}ClN_3O_2$
3o	-4Cl	-2,5-CH_3	6.5	222-224	68	$C_{24}H_{20}ClN_3O_2$
3p	-4Cl	-3,4-CH_3	9	257-259	70	$C_{24}H_{20}ClN_3O_2$

[a]Completion of the reaction was tested by the use of TLC. TLC: Thin-layer chromatography

1H, -NH-). MS (ESI) m/z=404.0 $(M+1)^+$; cald for, $C_{23}H_{19}N_3O_2$: 369.42. MS (ESI) m/z=404.0 $(M+1)^+$; cald for, $C_{23}H_{18}ClN_3O_2$: 403.86.

2-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-o-tolylacetamide (3e)

Yellow powder, IR (KBr, cm^{-1}): 3265 (N–H streching), 3214, 3055 (Ar–CH streching), 1734,1668 (C=O streching), 1602 (C=N streching), ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.204 (s, 3H, -CH_3), 4.671 (s, 2H,-CH_2), 6.534 (d, 1H, J=7.6 Hz, Ar-H), 6.881 (t, 1H, J=7.6, 7.6 Hz, Ar-H), 7.058-7.171 (m, 2H, Ar-H), 7.189-7.236 (m, 3H, Ar-H), 7.383 (d, 2H, J=8.8Hz, Ar-H), 7.469 (t, 1H, J=8, 7.6 Hz, Ar-H), 7.553 (d, 2H, J=8.8 Hz, Ar-H), 9.719 (s, 1H, -NH-). MS (ESI) m/z=405.1 $(M+2)^+$; cald for, $C_{23}H_{18}ClN_3O_2$: 403.86.

2-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-p-tolylacetamide (3f)

Brownish yellow powder, IR (KBr, cm^{-1}): 3308 (N–H streching), 3178, 2941 (Ar–CH streching), 1737, 1683 (C=O streching), 1603 (C=N streching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.255 (s, 3H, -CH_3), 4.632 (s, 2H, -CH_2), 6.528 (d, 1H, J=7.6 Hz, Ar-H), 6.879 (t, 1H, J=8, 7.6 Hz, Ar-H), 7.057-7.139 (m, 4H, Ar-H), 7.368-7.475 (m, 4H, Ar-H), 7.561 (d, 2H, J=8.4 Hz, Ar-H), 10.279 (s, 1H, -NH-). MS (ESI) m/z=403.9 $(M+1)^+$; cald for, $C_{23}H_{18}ClN_3O_2$: 403.86.

2-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(3-methoxyphenyl) acetamide (3g)

Yellow solid crystal, IR (KBr, cm^{-1}): 3309 (N–H stretching), 3157,3100 (Ar–CH stretching), 1743, 1687 (C=O stretching), 1606 (C=N stretching).^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.724 (s, 3H, -OCH_3-), 4.652 (s, 2H, -CH_2-), 6.460 (d, 1H, J=7.6 Hz, Ar-H), 6.655-6.680 (m, 1H, Ar-H), 6.876 (t, 1H, J=7.6, 8 Hz, Ar-H), 7.022 (t, 1H, J=8, 8 Hz, Ar-H), 7.079-7.165 (m, 2H, Ar-H), 7.173-7.264 (m, 2H, Ar-H), 7.336 (t, 2H, J=8, 12 Hz, Ar-H),7.438-7.477 (m, 1H, Ar-H), 7.529 (t, 1H, J=8, 7.6 Hz, Ar-H) 10.359 (s, 1H, -NH-). MS (ESI) m/z=420.0 $(M+1)^+$; cald for, $C_{23}H_{18}ClN_3O_3$: 419.86.

2-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(4-methoxyphenyl) acetamide (3h)

Brownish yellow powder, IR (KBr, cm^{-1}): 3292 (N–H stretching), 3067, 2935 (Ar–CH stretching), 1734, 1668 (C=O stretching), 1605 (C=N stretching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.722 (s, 3H, -OCH_3-),4.615 (s, 2H, -CH_2-), 6.445 (d, 1H, J=8 Hz, Ar-H), 6.889 (t, 3H, J=7.6, 8.8 Hz, Ar-H), 6.984-7.020 (m, 1H, Ar-H), 7.091-7.187 (m, 3H, Ar-H), 7.331-7.355 (m, 1H, Ar-H), 7.435-7.547 (m, 3H, Ar-H), 10.2 (s, 1H, -NH-). MS (ESI) m/z=420.0 $(M+1)^+$; cald for, $C_{23}H_{18}ClN_3O_3$: 419.86.

2-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(3-methoxyphenyl) acetamide (3i)

Redish yellow powder, IR (KBr, cm^{-1}): 3312 (N–H streching), 3198, 3064 (Ar–CH streching), 1733, 1696(C=O streching), 1607 (C=N streching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.716 (s, 3H, -OCH_3), 4.645 (s, 2H, -CH_2-), 6.528 (d, 1H, J=8 Hz, Ar-H), 6.638-6.674 (m, 1H, Ar-H), 6.878 (t, 1H, J=8, 7.2 Hz, Ar-H), 7.053-7.139 (m, 3H, Ar-H), 7.231 (t, 1H, J=8.4, 8.4 Hz, Ar-H), 7.301 (s, 1H, Ar-H), 7.376 (d, 1H, J=8.8 Hz, Ar-H), 7.449 (t, 1H, J=8, 7.6 Hz, Ar-H), 7.559 (d, 2H, J=8.4 Hz, Ar-H), 10.372 (s, 1H, -NH-). MS (ESI) m/z=420.0 $(M+1)^+$; cald for, $C_{23}H_{18}ClN_3O_3$: 419.86.

2-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(4-methoxyphenyl) acetamide (3j)

Yellow powder, IR (KBr, cm^{-1}): 3284 (N–H), 3064, 3012 (Ar–CH), 1737,1675 (C=O), 1603 (C=N), ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 3.721 (s, 3H, -OCH_3),4.610 (s, 2H, -CH_2-), 6.534 (d, 1H, J=7.6 Hz, Ar-H), 6.857-6.909 (m, 3H, Ar-H), 7.057-7.122 (m, 3H, Ar-H), 7.377 (d, 1H, J=8.8 Hz, Ar-H), 7.432-7.497 (m, 2H, Ar-H), 7.551 (t, 2H, J=8.8, 6.8 Hz, Ar-H), 10.195 (s, 1H, -NH-). MS (ESI) m/z=420.0 $(M+1)^+$; cald for, $C_{23}H_{18}ClN_3O_3$: 419.86.

2-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(2,4-dimethylphenyl) acetamide (3k)

Yellow solid powder, IR (KBr, cm^{-1}): 3274 (N–H stretching), 3064, 3032 (Ar–CH stretching), 1734, 1654 (C=O stretching), 1602 (C=N stretching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.156 (s, 3H, -CH_3-),

2.243 (s, 3H, -CH_3-), 4.645 (s, 2H, -CH_2-), 6.425 (d, 1H, J=7.6 Hz, Ar-H), 6.816 (t, 1H, J=8, 8 Hz, Ar-H), 6.958-7.163 (m, 3H, Ar-H), 7.206 (d, 2H, J=8 Hz, Ar-H), 7.263-7.30 (t, 1H, J=7.6, 7.2 Hz, Ar-H), 7.336 (t, 1H, Ar-H), 7.423-7.518 (m, 2H, Ar-H), 9.657 (s, 1H, -NH-). MS (ESI) m/z=418.0 $(M+1)^+$; cald for, $C_{24}H_{20}ClN_3O_2$: 417.89.

2-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(2, 5-dimethylphenyl) acetamide (3l)

Yellow solid powder, IR (KBr, cm^{-1}): 3283 (N–H stretching), 3049,3016 (Ar–CH stretching), 1739, 1669 (C=O stretching), 1604 (C=N stretching).^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.158 (s, 3H, -CH_3-), 2.243 (s, 3H, -CH_3-), 4.669 (s, 2H, -CH_2-), 6.453 (d, 1H, J=7.6 Hz, Ar-H), 6.868-6.942 (m, 2H, Ar-H), 7.006 (d, 1H, J=6.8 Hz, Ar-H), 7.080-7.204 (m, 4H, Ar-H), 7.358 (t, 1H, J=7.6, 6 Hz, Ar-H), 7.468-7.555 (m, 2H, Ar-H), 9.685 (s, 1H, -NH-). MS (ESI) m/z=418.0 $(M+1)^+$; cald for, $C_{24}H_{20}ClN_3O_2$: 417.89.

2-(3-(3-chlorophenylimino)-2-oxoindolin-1-yl)-N-(3,4-dimethylphenyl) acetamide (3m)

Yellow solid powder, IR (KBr, cm^{-1}): 3276 (N–H stretching), 3056, 2942 (Ar–CH stretching), 1730, 1683 (C=O stretching), 1602 (C=N stretching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.156 (s, 3H, -CH_3-), 2.243 (s, 3H, -CH_3-), 4.645 (s, 2H, -CH_2-), 6.425 (d, 1H, J=7.6 Hz, Ar-H), 6.816 (t, 1H, J=8, 8 Hz, Ar-H), 6.958-7.163 (m, 3H, Ar-H), 7.206 (d, 1H, J=8 Hz, Ar-H), 7.263-7.30 (t, 1H, J=7.6, 7.2 Hz, Ar-H),7.336 (t, 1H, Ar-H), 7.423-7.518 (m, 3H, Ar-H), 9.657 (s, 1H,-NH-). MS (ESI) m/z=417.9 $(M+1)^+$; cald for, $C_{24}H_{20}ClN_3O_2$: 417.89.

2-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(2,4-dimethylphenyl) acetamide (3n)

Orange powder, IR (KBr, cm^{-1}): 3273, (N–H streching), 3048, 2966 (Ar–CH streching), 1730,1688.(C=O streching), 1604 (C=N streching), ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.157 (s, 3H, -CH_3), 2.249 (s, 3H, -CH_3), 4.651 (s, 2H, -CH_2-), 6.532 (d, 1H, J=7.2 Hz, Ar-H), 6.887 (t, 1H, J=8, 7.2 Hz, Ar-H), 6.976 (d, 1H, J=8.8 Hz, Ar-H), 7.025-7.138 (m, 3H, Ar-H), 7.208 (d, 1H, J=8.4 Hz, Ar-H), 7.391 (d, 1H, J=8.4 Hz, Ar-H), 7.475 (t, 1H, J=7.6, 8 Hz, Ar-H), 7.573 (d, 2H, J=8 Hz, Ar-H) 9.672 (s, 1H, -NH-). MS (ESI) m/z=418.0 $(M+1)^+$; cald for, $C_{24}H_{20}ClN_3O$: 417.89.

2-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(2, 5-dimethylphenyl) acetamide (3o)

Shiny yellow crystal, IR (KBr, cm^{-1}): 3264 (N–H streching), 3064, 3031 (Ar–CH sterching), 1734, 1669 (C=O sterching), 1609 (C=N streching). ^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.151 (s, 3H, -CH_3), 2.236 (s, 3H, -CH_3), 4.661 (s, 2H, -CH_2-), 6.528 (d, 1H, J=7.6 Hz, Ar-H), 6.866-6.935 (m, 2H, Ar-H), 7.060-7.138 (m, 3H, Ar-H), 7.183 (s, 1H, Ar-H), 7.389 (d, 1H, J=8.8 Hz, Ar-H), 7.476 (t, 1H, J=8, 7.6 Hz, Ar-H), 7.560 (d, 2H, J=8.4 Hz, Ar-H), 9.683 (s, 1H, -NH-). MS (ESI) m/z=418.0 $(M+1)^+$; cald for, $C_{24}H_{20}ClN_3O$: 417.89.

2-(3-(4-chlorophenylimino)-2-oxoindolin-1-yl)-N-(3, 4-dimethylphenyl) acetamide (3p)

Yellow powder, IR (KBr, cm^{-1}): 3291 (NH streching), 3066, 2940 (Ar–CH streching), 1730, 1683 (C=O streching), 1606 0 (C=N streching).^{1}H NMR (400 MHz, δ, ppm, DMSO-d_6): 2.164 (s, 3H, -CH_3), 2.180 (s, 3H, -CH_3), 4.619 (s, 2H, -CH_2-), 6.526 (d, 1H, J=7.6 Hz, Ar-H), 6.876 (t, 1H, J=8, 7.6 Hz, Ar-H), 7.037-7.123 (m, 4H, Ar-H), 7.278-7.322 (m, 1H, Ar-H), 7.445 (t, 1H, J=8, 8 Hz, Ar-H), 7.366 (t, 1H, J=9.2, 2 Hz, Ar-H), 7.561(d, 2H, J=8.8 Hz, Ar-H), 10.194 (s, 1H, -NH-). MS (ESI) m/z=418.0 $(M+1)^+$; cald for, $C_{24}H_{20}ClN_3O$: 417.89.

Biological studies

Anthelmintic activity

African adult earth worm 4-5 cm in length and 0.1-0.2 cm in width were used for all the newly synthesized prototypes. All the earthworms were collected from Divyayan Krishi Vigyan Kendra, Morabadi, Ranchi, Jharkhand, India. The worms were divided into the different groups containing six-earth worms in each group. All the prototypes were dissolved in minimum quantity of 2% v/v Tween 80 and the volume was adjusted to 10 ml with normal saline for assembly the concentration of 5, 10, 20 mg/ml. Before initiation of the experiments, all the prototypes, and standard drug solution were freshly prepared. All the earthworms were washed in normal saline solution before they were released into 10 ml of respective formulation as follows, vehicle (2% v/v Tween 80 in normal saline), albendazole (20 mg/ml) and prototypes (5, 10, 20 mg/ml). The anthelmintic activity was determined in six observations were followed for six worms regarding the same size per petridish were used. They were pragmatic for their natural motility and evoked responses. Observations were made for the time taken to paralysis and death of individual worms. Paralysis was said to occur when the worms did not revive even in normal saline. Death was concluded when worms lost their motility followed with fading away of their body color [31].

Statistical analysis

Results were expressed as mean ± standard deviation. Statistical significance was determined by one-way analysis of variance followed by Dennett's test, with the level of significance at $p<0.01$ and $p<0.05$.

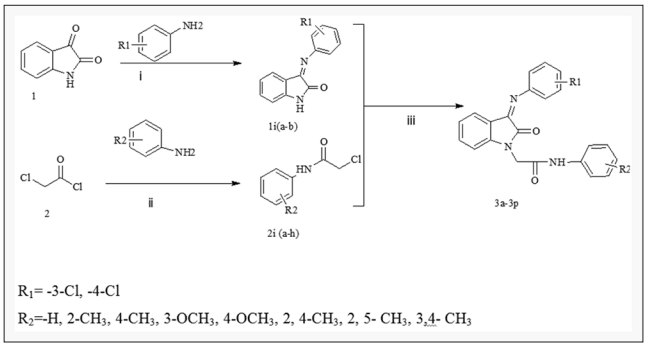
RESULTS AND DISCUSSION

Chemistry

In the present study, a small library of 16 isatin analogs (3a-3p) was synthesized following the reaction outlined in Scheme 1. The synthesis of the title compounds was realized in three steps.

First, 1, 3-dihydro-indol-2-ones (1_ia-b) were synthesized (Schiff base) following the method reported for isatins [26,27]. For Schiff base reaction, isatin (1) and substituted anilines were dissolved in warm ethanol in the presence of 2-3 drops of glacial acetic acid and refluxed for 2-3 hrs. After standing for approximately 24 hrs at room temperature (r.t), the yellow crystalline products were separated by filtration, vacuum dried, and recrystallized from ethanol. Next, substituted anilines and 2-chloroacetyl chloride (2) were reacted in the presence of glacial acetic acid in ice-cold condition leading to the formation of chloroanilides (2_ia-h) following the procedure reported by Soyer *et al*. Finally, the chloroanilides (2_ia-h) were treated with 1, 3-dihydro-indol-2-ones (1_ia-b) to yield the title compounds (3a-3p) [25].

The IR (KBr) spectra of all the synthesized compounds exhibited very similar frequencies indicating the presence of amide structures for the title compounds. The IR spectra of the synthesized compounds afforded the acetamide carbonyl stretching (C=O) band 1731-1607/cm. Similarly, N-H stretching band of the amide was seen between 3400 and 3300/cm. The C=N band in IR spectra of all the compounds appeared at 1605-1550/cm respectively which is similar as that of the ordinary C=N absorption. The ^{1}H NMR spectrum of the title compounds (3a-3p) were recorded in DMSO-d_6 solution and are in entire concord with the expected resonance signals in terms of chemical shifts and



Scheme 1: Reagents and conditions: (i) Ethanol, 2-3 drops glacial acetic acid, reflux, 2-3 hrs, (ii) glacial acetic acid, cold condition, 1 hr, (iii) dimethylformamide, KI, heating at 60°C, 2-12 hrs

Table 2: Anthelmintic activity of synthesized compounds (3a-3p) *against Pheretima Posthuma*

Compound	Time taken for paralysis (P) and death (D) (mean±SD)					
	Paralysis time (minutes)			Lethal time (minutes)		
	5 mg/ml	10 mg/ml	20 mg/ml	5 mg/ml	10 mg/ml	20 mg/ml
3a	28.57±0.73	23.26±0.85	10.43±0.05	32.56±0.4	26.9±0.62	15.23±0.15
3b	21.87±0.51	17.96±0.51	8.26±0.12	28.6±0.36	24±0.55	10.2±0.2
3c	23.94±0.47	15.06±0.49	5.93±0.56	30.86±0.61	24.53±0.75	10.96±0.49
3d	24.64±0.68	20.96±0.41	12.67±0.66	36.73±0.66	29±0.45	20.26±0.15
3e	26.67±0.47	22.63±0.58	9.3±0.17	30.56±0.47	25.96±0.58	13.33±0.15
3f	12.6±0.62	9.63±0.49	5.96±0.58	22.6±0.55	18.56±0.47	8.96±0.61
3g	14.06±0.51	9.03±0.56	5.26±0.8	17.03±0.56	12.63±0.41	7.03±0.43
3h	15.57±0.43	11.63±0.41	6.03±0.5	20.66±0.47	16.26±0.85	8.93±0.47
3i	15±0.45	10.12±0.41	5.93±0.57	17.83±0.68	13.66±0.37	8.7±0.6
3j	18±0.45	13.96±0.41	7.93±0.47	28.73±0.58	19.66±0.37	11.7±0.6
3k	20.33±0.76	16.03±0.5	4.9±0.56	24.6±0.55	19.93±1.11	6.7±0.56
3l	22.23±0.96	17.7±0.62	6.35±0.07	25.3±0.95	20.63±0.49	8.7±0.56
3m	20.94±0.41	15.34±0.43	5.26±0.2	24.53±0.41	18.93±0.47	7.75±0.1
3n	20±0.7	15.67±0.56	5.3±0.28	30.1±0.6	21.7±0.7	11.85±0.63
3o	25.93±0.56	22.63±0.41	12.67±0.66	32.87±0.7	28.96±0.45	17.03±0.47
3p	23±0.7	20.06±0.52	8.06±0.58	26.76±0.64	22.63±0.58	10.26±0.06
Albendazole			5.43±0.34			7.52±0.36

All determinations were done in triplicate and results are expressed as mean±SD. p value was calculated by comparing with control by one-way ANOVA. Control worms were alive up to 24 hrs of observation. Albendazole were used at 20 mg/ml. SD: Standard deviation

integrations.[1]H NMR spectrum of the title compounds (3a-3p) showed a broad singlet of 1 proton assigned to NH proton at δ 10.96-10.82. Depending on the substitution patterns on the N -phenyl ring, the aromatic protons of certain compounds (3a-3p) also showed distinct chemical shifts with expected splitting patterns as doublets, triplets, or multiplets integrating more than one proton due to the close chemical shifts ranging from δ 6.365 to 7.582. In the aliphatic region, a broad singlet of 2 protons assigned to the methylenic proton of N-CH_2-CO at range δ 4.613-4.676 was observed for the compounds (3a-3p). A broad singlet of 3 protons assigned to the methoxy protons of -OCH_3 at δ 3.710, 3.717, 3.727 and 3.722 was observed at the second phenyl ring of the compounds 3g, 3h, 3i, and 3j, respectively. A broad singlet of 3 protons assigned to methyl protons of -CH_3 at δ 1.993-2.286 was observed at the second phenyl ring of the compounds 3c, 3d, 3e, 3f, 3k, 3l, 3m, 3n, 3o, and 3p. The NMR data of the title compounds (3a-3p) are summarized in experimental sections. The structural confirmations of these compounds were determined by using ESI-MS. The physicochemical data are presented in Table 1.

Biological activity

The synthesized compounds (3a-3p) were evaluated for their *in vitro* anthelmintic activity against *Pheretima posthuma* [32,33]. Albendazole was used as a standard drug at a dose of 20 mg/ml. The dose used for the newly synthesized compounds were 5, 10 and 20 mg/ml. The mean paralysis time and lethal time were premeditated for each test compound and a standard drug (each reading was taken in triplicate). The results are depicted in Table 2.

All the synthesized compounds (3a-3p) at a minimal dose of 5 mg/ml exhibited anthelmintic activity. All the test compounds showed a comparative significant reduction ($p<0.01$) and ($p<0.05$) in time taken for paralysis and death as compared to control at the dose level of 5 mg, 10 mg and 20 mg. It is apparent that all the synthesized compounds exhibited significant anthelmintic activity ($p<0.01$) and ($p<0.05$) in a dose-dependent manner giving shortest time of paralysis and death at 20 mg/ml concentration.

It was observed that time taken for paralysis and death was least for test compound 3f and 3i at dose level of 5 mg and was highly significant compared to that of the standard albendazole at a dose of 20 mg/ml. It was interesting to observe that of the remaining synthesized compounds also showed significant activity compared to standard albendazole. Paralytic and lethal time was slightly less than that of standard albendazole at the same dose level.

Structure-activity relationship

Studies suggested that compounds with hydrophobic and electron withdrawing/electron releasing groups substituted in the phenyl rings flanking the isatin moiety showed the highest anthelmintic activity. In fact, when a chloro group was substituted at the *para* position of the first phenyl ring with a methoxy group substituted at the third position (meta position) of the 2nd phenyl ring attached to the isatin moiety by a CH_2CONH linker, significant activity was exhibited as in case of compound 3i. Similar pattern of activity was observed compound 3f when the methoxy group was replaced to the methyl group and shifted to the 4th position (*para* position) of the phenyl ring. The absence of any substitution in the second phenyl ring was detrimental to activity as observed in the case of compounds 3a and 3b. Even with incorporation of more than one methyl group activity did not rise, and there was no significant change in activity. Thus, hydrophobicity and the position of halogen group in the first phenyl ring were highly important for an increase in anthelmintic activity.

CONCLUSION

We have synthesized some isatin analogs (3a-3p) and evaluated these compounds for their anthelmintic activities, and their structures have been characterized by IR, NMR and mass spectroscopy for developing better anthelmintic molecules. The simple isatin analogs 3f and 3i were the most potent derivatives in all the cases. The study encourages us to consider a new molecular skeleton of isatin substituted at the first and third position by aryl groups with adequate spacers which may be identified as a potential leads for the development of future studies in various *in vivo* models for anthelmentic activities.

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