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REGULAR ARTICLE

Design and synthesis of novel isatin derivatives as potent analgesic, anti-inflammatory and antimicrobial agents

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Abstract. A powerful analgesic, anti-inflammatory and antimicrobial drug was developed by synthesizing a set of new indole-2,3-dione derivatives. The chemical structures of the synthesized derivatives were confirmed by spectroscopic and elemental analyses. Tail flick method, carrageenan-induced foot paw edema technique and agar streak dilution methods were employed to evaluate analgesic, anti-inflammatory and antimicrobial activities (*in vitro*) of drugs, respectively. In addition, ulcerogenicity of potent derivatives was also estimated. Most of the synthesized derivatives displayed low-to-reasonable ulcer index, with mild-to-good analgesic, anti-inflammatory and antimicrobial potency. The SARs between the biological activity and differences in the functional group of the title compounds have been explained. 1-(4-Chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)thiosemicarbazide **VIc** has been found as the potent derivative of this series.

Keywords. Isatin; thiosemicarbazide; analgesic; anti-inflammatory; antimicrobial activity.

1. Introduction

Universally nonsteroidal anti-inflammatory drugs (NSAIDs) are one of the most important common healing groups of drugs employed for the management of fever, pain and inflammation.[1] In contrast, the efficiency of NSAIDs was narrowed due to increased occurrence of the sensible gastrointestinal (GI) damage primarily ulceration of GI and its connected perforation and complications.[2] Two dissimilar mechanisms are the reason for these main side effects. The first one involves ion trapping mechanism by local effect consists of direct contact consequences which results from the acidic atmosphere of these agents and their effect under the local neutral or moderately acidic state of the stomach. The second succeeding mechanism is NSAIDs induced gastropathy due to the widespread systemic effect after its absorption. This effect is linked to their intrinsic effect on *COX (cyclooxygenase)* enzyme. The latter one is accountable for its preferred anti-inflammatory potency.

There are lots of key breakthroughs observed during the past few decades in many areas of modern drug. Even though, a major problem over the last 10 years was treatment against multidrug-resistant pathogens which remains a main challenge. In general these microbial infections are associated along with fever, pain and inflammation. Outstanding drugs are available worldwide for treating these conditions individually. However, in the market no single drug was available to cure all these three problems. Hence, in this research our major aim was to discover a new derivative possessing all these biological properties (analgesic, anti-inflammatory and antimicrobial) with less toxicity. From the literature review, it found that isatin and its derivatives particularly Schiff and Mannich bases possess all these aforementioned properties.[3–7]

Due to its attractive pharmacological effects, isatin has gained an enormous attention from medicinal chemists.[8–21] This was confirmed in 1965, from the finding of metisazone (isatin-2,3-dione based compound) an antiviral agent used against viral infections as prophylactic agent (Figure 1). Additionally, literature suggests that Mannich and Schiff bases of isatin

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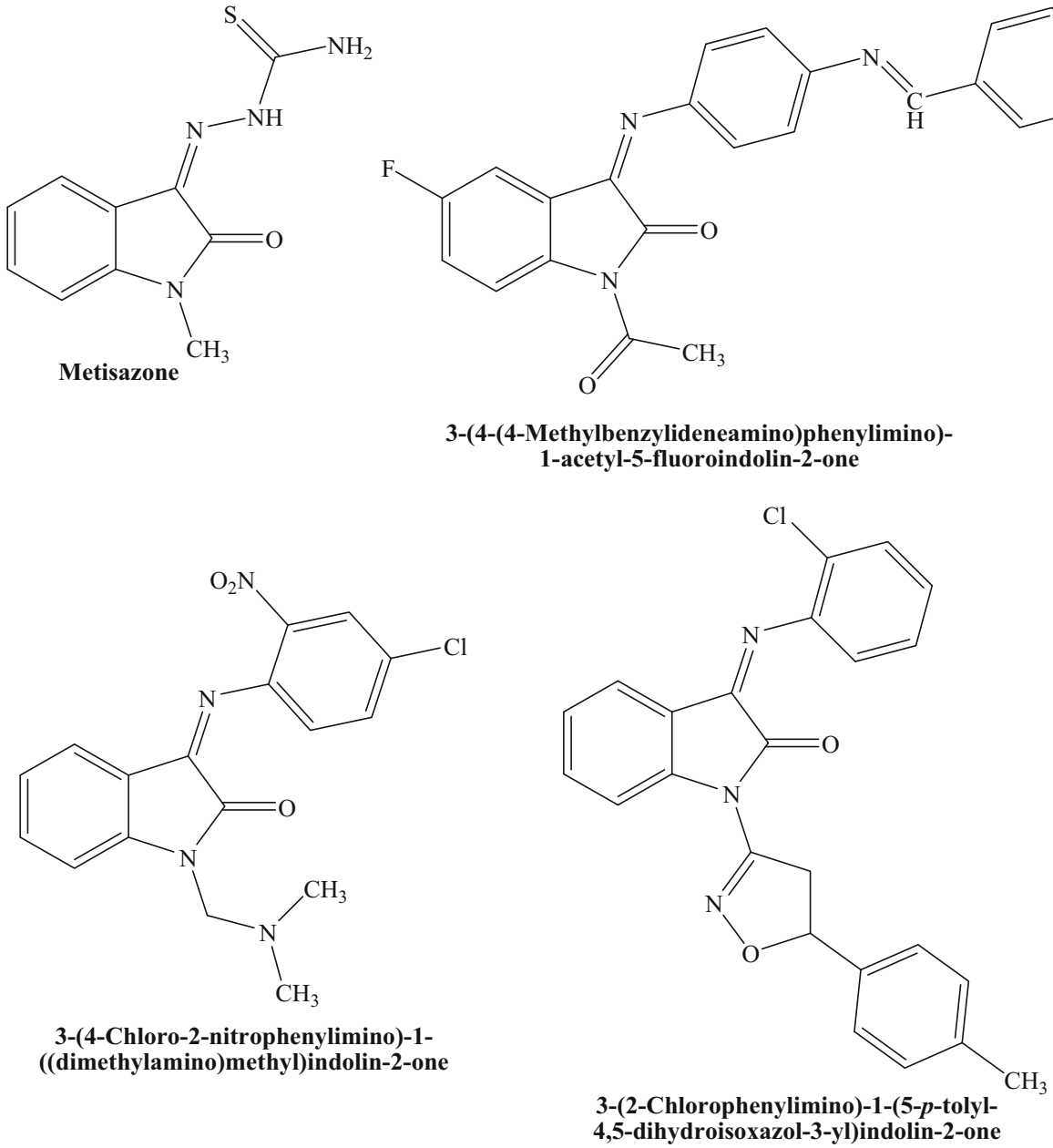


Figure 1. Isatin with potent pharmacological activity.

ring clearly improved antimicrobial potencies.[22,23] In addition, isatin ring was also reported to possess powerful analgesic and anti-inflammatory properties.[24,25] Various potent analgesic and anti-inflammatory agents possessing isatin ring are presented in Figure 1.

Mannich and Schiff bases obtained from several heterocyclic derivatives gained significance due to their significant pharmacological and physiological properties. Based on these, it aimed to synthesize novel isatin derivatives as Mannich and Schiff bases as possible antimicrobial, analgesic, and anti-inflammatory drugs. The concept of target molecule design was presented in Figure 2. Development of ulcer was one of the major disadvantages of all existing analgesic and anti-inflammatory drugs. As a result, ulcerogenic potential of the most active title compounds was also evaluated.

2. Experimental

2.1 *Materials*

Melting points (mp) are measured in open capillaries and are uncorrected on a Thomas Hoover melting point

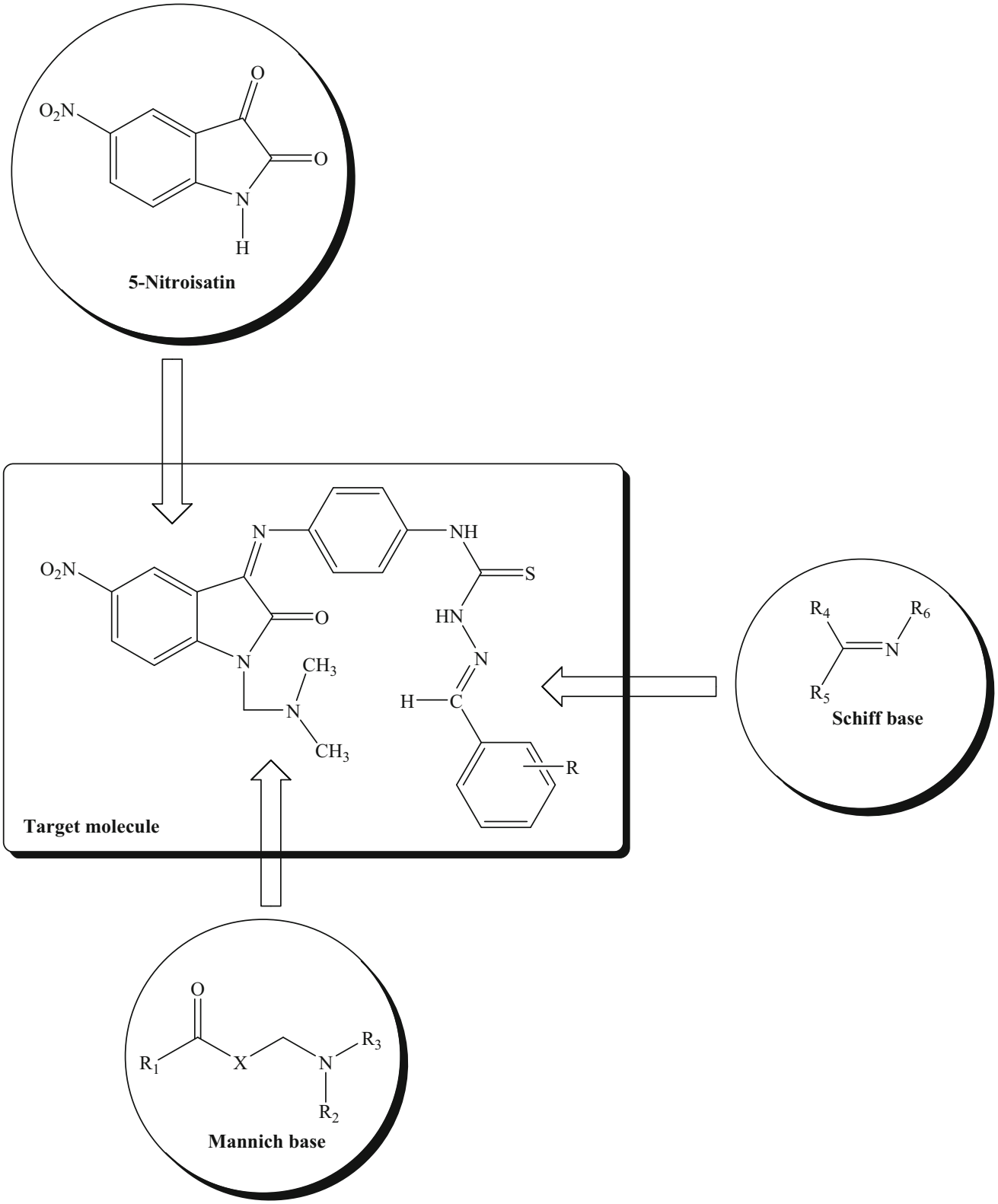


Figure 2. The design concept of the target molecule (**VIa–VIm**).

apparatus. On Bruker FT-IR spectrometer the IR spectra (ν, cm^{-1}) were measured using KBr disks. At 300 MHz ^{1}H-NMR spectra were recorded in $CDCl_3$ using a Bruker FT-NMR spectrometer in ppm (parts per million, δ) using internal standard TMS (tetramethylsilane). Similarly at 125 MHz, ^{13}C-NMR spectra were recorded using a Bruker FT-NMR spectrometer in ppm (parts per million, δ) using $CDCl_3$ as internal standard. Using FAB (fast

atom bombardment) positive in a JEOL-SX-102 instrument, mass spectra were recorded. Elemental analysis was measured on a PerkinElmer 2400 CHN analyzer. Experimental values are compared against calculated values and were found to be within the satisfactory confines (± 0.4 %). Using readymade silica gel plates the reaction progresses were monitored. UV lamp and iodine were used as developing agent to detect the compounds. In this study the entire chemicals and reagents used were obtained from Qualigens, CDH, SD Fine Chem. and E. Merck India Ltd. and were used without additional purification.

2.2 *Preparation of 5-nitroisatin (**I**)*

Derivative **I** was synthesized according to the protocol reported in the literature.[26] Briefly, to a solution of concentrated H_2SO_4 (0.75 mol; 73.4 g) and concentrated HNO_3 (0.50 mol; 31.5 g) isatin (0.33 mol; 48.51 g) was added slowly with frequent shaking in RBF (500 mL). Ice cold water was used to cool the obtained solution by immersing the flask in it. Reflux condenser was fixed in association with flask, after adding all isatin. At 60 °C on water bath, the reaction mixtures were refluxed for a period of 1 h to produce the preferred derivative 5-nitroisatin **1**. Then whole solution was added to cold water (500 mL) contained in beaker to remove as much acid from 5-nitroisatin **1**. From the mixture the upper acid layer was removed when derivative **I** was settled completely at the bottom. Subsequently, the base layer was transferred to the separating funnel containing water (50 mL) and shaken vigorously. Finally, the solid separated was collected and dehydrated with calcium chloride (anhydrous) in order to get compound **I** in pure form. Yield: 65 %; M.p. 230–232 °C. IR (KBr cm^{-1}): 3342 (NH), 2996 (Ar-CH), 1732 (C=O), 1570 and 1348 (NO_2). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 7.02–7.94 (m, 3H, Ar-H), 8.92 (s, 1H, NH of isatin). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 188.4 (C-3), 157.8 (C-2), 155.0 (C-8), 140.9 (C-5), 130.3 (C-6), 124.1 (C-4), 121.6 (C-7), 114.2 (C-9). EI-MS *m/z*: 192. *Anal*. Calcd for $C_8H_4N_2O_4$: C, 50.01; H, 2.10; N, 14.58. Found: C, 49.91; H, 2.11; N, 14.62.

2.3 *Preparation of 3-(4-aminophenylimino)-5-nitroindolin-2-one (**II**)*

4-Amino benzoic acid (0.01 mol; 1.37 g) and 5-nitroisatin (0.01 mol; 1.92 g) was mixed in a RBF containing ethanol (25 mL) and glacial acetic acid (few drops). At 100 °C in a water bath, the above mixture was refluxed for a period of 3 h. The obtained solution was kept at room temperature until it cool and the resulting compound was collected. The collected compound **II** was washed with ethanol and re-crystallized using chloroform and ethanol mixture. Yield: 76 %; M.p. 189–192 °C. IR (KBr cm^{-1}): 3353 and 3281 (NH), 3038 (Ar-CH), 1702 (C=O), 1640 (C=N), 1634 (C=C), 1515 and 1349 (NO_2). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 4.37 (s, 2H, NH_2), 7.19–8.32 (m, 7H, Ar-H), 8.95 (s, 1H, NH of isatin). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 169.5 (C-2), 166.1 (C-3), 150.7 (C-8), 148.9 (C-1′), 144.2 (C-5), 142.9 (C-4′), 127.4 (C-4), 125.8 (C-6), 125.3 (C-2′ and C-6′), 123.0 (C-7), 116.6 (C-9), 114.2 (C-3′ and C-5′). EI-MS *m/z*: 282. *Anal*. Calcd for $C_{14}H_{10}N_4O_3$: C, 59.57; H, 3.57; N, 19.85. Found: C, 59.74; H, 3.56; N, 19.81.

2.4 *Preparation of 3-(4-aminophenylimino)-1-((dimethylamino)methyl)-5-nitroindolin-2-one (**III**)*

37 % Aqueous formaldehyde (0.25 mL) was added to a mixture containing ethanol (25 mL) and 3-(4-aminophenylimino)-5-nitroindolin-2-one **II** (0.01 mol; 2.82 g) at once. To the above mixture, dimethylamine (0.04 mol; 1.8 g) was added portion wise with slow stirring. After adding all dimethylamine, the reaction mixture was stirred mechanically for the period of 6 h at room temperature and then set aside for 48 h in refrigerator to obtain product. Lastly, the formed crystal was separated by filtration and dried in vacuum. In order to get desired products in pure form, the crystal was re-crystallized from alcohol. Yield: 70 %; M.p. 235–237 °C. IR (KBr cm^{-1}): 3389 and 3305 (NH), 3013 (Ar-CH), 2976 (CH_3-CH), 1732 (C=O), 1654 (C=N), 1621 (C=C), 1547 and 1320 (NO_2). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.59 (s, 6H, $N(CH_3)_2$), 4.04 (s, 2H, NH_2), 4.17 (s, 2H, CH_2 linkage), 7.02–8.13 (m, 7H, Ar-H). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 168.6 (C-2), 167.2 (C-3), 154.0 (C-8), 146.1 (C-1′), 145.9 (C-5), 144.5 (C-4′), 126.7 (C-4), 125.3 (C-6), 125.0 (C-2′ and C-6′), 123.8 (C-7), 119.4 (C-9), 116.7 (C-3′ and C-5′), 78.2 (CH_2 linkage), 40.9 ($N(CH_3)_2$). EI-MS *m/z*: 339. *Anal*. Calcd for $C_{17}H_{17}N_5O_3$: C, 60.17; H, 5.05; N, 20.64. Found: C, 59.99; H, 5.07; N, 20.71.

2.5 *Synthesis of methyl 4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl carbamodithioate (**IV**)*

A solution containing 3-(4-aminophenylimino)-1-((dimethylamino)methyl)-5-nitroindolin-2-one **III**

(0.01 mol; 3.39 g) and DMSO (10 mL) was vigorously stirred in a magnetic stirrer. To the above solution, 20 M aqueous sodium hydroxide (0.6 mL) and carbon disulphide (0.01 mol; 0.75 g) was added drop wise, with stirring for duration of 30 min. Then, obtained solution was immediately kept in freezing mixture and dimethyl sulphate (0.01 mol; 1.26 g) was added gradually to the formed intermediate i.e., [sodium-4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenylcarbamodithioate] with stirring. The mechanical stirring was continued for further 6 h period. Finally, the above obtained solution was transferred into ice cold water with vigorous stirring. The product separated **IV** was filtered and washed with water. Dried and re-crystallized the product from ethanol. Yield = 72 %; M.p. 174–175 °C. IR (KBr) cm^{-1}: 3384 (NH), 3010 (Ar-CH), 2968 (CH_3-CH), 1733 (C=O), 1659 (C=N), 1602 (C=C), 1529 and 1337 (NO_2), 1261 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 1.88 (s, 3H, SCH_3), 2.41 (s, 6H, $N(CH_3)_2$), 4.25 (s, 1H, CH_2 linkage), 7.23–8.10 (m, 7H, Ar-H), 9.16 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 195.4 (C=S), 165.7 (C-2), 165.3 (C-3), 154.9 (C-8), 151.5 (C-1′), 143.2 (C-5), 137.1 (C-4′), 126.4 (C-3′ and C-5′), 123.8 (C-4), 123.3 (C-6), 122.0 (C-2′ and C-6′), 121.5 (C-7), 117.9 (C-9), 74.2 (CH_2 linkage), 41.9 ($N(CH_3)_2$), 24.6 (SCH_3). EI-MS *m/z*: 429 (M^+). *Anal*. Calcd for $C_{19}H_{19}N_5O_3S_2$: C, 53.13; H, 4.46; N, 16.31. Found: C, 53.30; H, 4.47; N, 16.26.

2.6 *Synthesis of 4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)thiosemi carbazide (**V**)*

Hydrazine hydrate (0.01 mol; 0.5 g) and the above synthesized methyl 4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenylcarbamodithioate **IV** (0.01 mol; 4.29 g), was added to RBF containing ethanol (30 mL). Anhydrous potassium carbonate (100 mg) was added to this mixture and refluxed in water bath for 24 h. Obtained solution was cooled and the solid separated was filtered. Using 10 % sodium hydroxide solution (alcoholic), the product was purified by dissolving in it and re-precipitated using dilute HCl. The compound separated was filtered and washed with water. Finally, dried and re-crystallized the compound **V** using ethanol to get pure compound **V**. Yield = 79 %; M.p. 151–153 °C. IR (KBr) cm^{-1}: 3351 and 3298 (NH), 3045 (Ar-CH), 2994 (CH_3-CH), 1700 (C=O), 1646 (C=N), 1603 (C=C), 1552 and 1314 (NO_2), 1279 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.14 (s, 2H, NH_2), 2.40 (s, 6H, $N(CH_3)_2$), 4.08 (s, 2H, CH_2 linkage), 6.91–8.03 (m, 7H, Ar-H), 8.76 (s, 1H, CSNH), 9.25 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 172.9 (C=S), 162.4 (C-2), 162.1 (C-3), 154.7 (C-8), 148.5 (C-1′), 144.2 (C-5), 136.8 (C-4′), 129.6 (C-3′ and C-5′), 125.0 (C-4), 124.7 (C-6), 123.1 (C-2′ and C-6′), 122.9 (C-7), 116.5 (C-9), 77.3 (CH_2 linkage), 44.8 ($N(CH_3)_2$). EI-MS *m/z*: 413 (M^+). *Anal*. Calcd for $C_{18}H_{19}N_7O_3S$: C, 52.29; H, 4.63; N, 23.71. Found: C, 52.16; H, 4.65; N, 23.77.

2.7 *Synthesis of 1-(substituted benzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylidene amino)phenyl)thiosemicarbazide (**VIa–VIm**)*

To a finely mixed solution of ethanol (30 mL), glacial acetic acid (0.5 mL) and various aromatic aldehydes (0.01 mol), 4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)thiosemicarbazide **V** (0.01 mol; 4.13 g) was added portion wise with stirring. The obtained solution was refluxed on water bath over night and set aside for some time. Latter the mixture was transferred into ice cold water with stirring. The compound that separated out **VIa–VIm** was filtered and dried. Ethanol was used to re-crystallize the compounds. Spectral analyses evidences that the technique employed for the synthesis and separation produced good quality of compounds.

2.7a *1-Benzylidene-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)thiosemicarbazide* (**VIa**): Yield = 75 %; M.p. 245–247 °C. IR (KBr) cm^{-1}: 3373 and 3217 (NH), 3049 (Ar-CH), 2980 (CH_3-CH), 1714 (C=O), 1662 (C=N), 1629 (C=C), 1535 and 1323 (NO_2), 1274 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.43 (s, 6H, $N(CH_3)_2$), 4.20 (s, 2H, CH_2 linkage), 7.19–8.36 (m, 12H, Ar-H), 8.84 (s, 1H, N=CH), 9.37 (s, 1H, CSNH), 9.82 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 183.7 (C=S), 164.4 (C-2), 163.2 (C-3), 153.5 (C-8), 150.8 (C-1′), 146.1 (C-5), 144.7 (N=CH), 138.4 (C-4′), 134.3 (C-1″), 132.0 (C-4″), 129.8 (C-2″ and C-6″), 129.4 (C-3″ and C-5″), 128.6 (C-3′ and C-5′), 126.0 (C-4), 124.3 (C-6), 120.9 (C-2′ and C-6′), 119.1 (C-7), 116.5 (C-9), 74.6 (CH_2), 45.0 ($N(CH_3)_2$). EI-MS *m/z*: 501 (M^+). *Anal*. Calcd for $C_{25}H_{23}N_7O_3S$: C, 59.87; H, 4.62; N, 19.55. Found: C, 60.08; H, 4.60; N, 19.51.

2.7b *1-(4-Nitrobenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) thiosemicarbazide* (**VIb**): Yield = 78 %; M.p. 284–286 °C. IR (KBr) cm^{-1}: 3325 and 3242 (NH), 3038 (Ar-

CH), 2983 (CH_3-CH), 1726 (C=O), 1659 (C=N), 1618 (C=C), 1524 and 1346 (NO_2), 1247 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.30 (s, 6H, $N(CH_3)_2$), 4.14 (s, 2H, CH_2 linkage), 7.06–8.12 (m, 11H, Ar-H), 8.57 (s, 1H, N=CH), 9.21 (s, 1H, CSNH), 10.19 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 181.3 (C=S), 160.1 (C-2), 159.5 (C-3), 153.7 (C-8), 151.5 (C-4″), 148.4 (C-1′), 145.2 (C-5), 143.5 (N=CH), 142.8 (C-1″), 134.2 (C-4′), 131.5 (C-2″ and C-6″), 128.7 (C-3′ and C-5′), 125.9 (C-4), 124.0 (C-6), 123.8 (C-2′ and C-6′), 123.4 (C-7), 120.2 (C-3″ and C-5″), 119.6 (C-9), 77.2 (CH_2), 43.4 ($N(CH_3)_2$). EI-MS *m/z*: 546 (M^+). *Anal*. Calcd for $C_{25}H_{22}N_8O_5S$: C, 54.94; H, 4.06; N, 20.50. Found: C, 55.12; H, 4.05; N, 20.43.

2.7c *1-(4-Chlorobenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) thiosemicarbazide* (**VIc**): Yield = 80 %; M.p. 260–262 °C. IR (KBr) cm^{-1}: 3338 and 3270 (NH), 3033 (Ar-CH), 2968 (CH_3-CH), 1736 (C=O), 1654 (C=N), 1617 (C=C), 1521 and 1328 (NO_2), 1265 (C=S), 789 (C-Cl). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.15 (s, 6H, $N(CH_3)_2$), 4.52 (s, 2H, CH_2 linkage), 6.94-7.90 (m, 11H, Ar-H), 8.61 (s, 1H, N=CH), 9.29 (s, 1H, CSNH), 9.66 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 179.8 (C=S), 160.0 (C-2), 158.9 (C-3), 156.1 (C-8), 153.7 (C-1′), 142.8 (C-5), 141.3 (N=CH), 137.1 (C-4″), 134.5 (C-4′), 130.7 (C-1″), 130.2 (C-2″ and C-6″), 129.5 (C-3″ and C-5″), 125.2 (C-3′ and C-5′), 122.6 (C-4), 121.3 (C-6), 120.8 (C-2′ and C-6′), 118.4 (C-7), 117.6 (C-9), 76.4 (CH_2), 47.7 ($N(CH_3)_2$). EI-MS *m/z*: 537 (M^{+2}), 535 (M^+). *Anal*. Calcd for $C_{25}H_{22}ClN_7O_3S$: C, 56.02; H, 4.14; N, 18.29. Found: C, 56.19; H, 4.13; N, 18.32.

2.7d *1-(4-Methylbenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide* (**VId**): Yield = 75 %; M.p. 222–224 °C. IR (KBr) cm^{-1}: 3394 and 3350 (NH), 3026 (Ar-CH), 2978 (CH_3-CH), 1715 (C=O), 1667 (C=N), 1626 (C=C), 1530 and 1309 (NO_2), 1252 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.27 (s, 6H, $N(CH_3)_2$), 2.74 (s, 3H, CH_3), 4.31 (s, 2H, CH_2 linkage), 6.85-7.97 (m, 11H, Ar-H), 8.80 (s, 1H, N=CH), 9.36 (s, 1H, CSNH), 9.83 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 178.5 (C=S), 164.8 (C-2), 162.4 (C-3), 155.0 (C-8), 150.6 (C-1′), 148.9 (C-5), 144.2 (N=CH), 138.3 (C-4″), 136.0 (C-4′), 131.8 (C-1″), 130.6 (C-3″ and C-5″), 130.2 (C-2″ and C-6″), 127.5 (C-3′ and C-5′), 123.4 (C-4), 122.7 (C-6), 121.2 (C-2′ and C-6′), 120.1 (C-7), 115.3 (C-9), 74.7 (CH_2), 42.1 ($N(CH_3)_2$), 23.8 (CH_3). EI-MS *m/z*: 515 (M^+). *Anal*. Calcd for $C_{26}H_{25}N_7O_3S$: C, 60.57; H, 4.89; N, 19.02. Found: C, 60.38; H, 4.90; N, 19.09.

2.7e *1-(4-Methoxybenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide* (**VIe**): Yield = 73 %; M.p. 258–260 °C. IR (KBr) cm^{-1}: 3301 and 3234 (NH), 3020 (Ar-CH), 2979 (CH_3-CH), 1722 (C=O), 1648 (C=N), 1623 (C=C), 1535 and 1338 (NO_2), 1289 (C=S), 1013 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.28 (s, 6H, $N(CH_3)_2$), 3.91 (s, 3H, OCH_3), 4.46 (s, 2H, CH_2 linkage), 7.08-8.29 (m, 11H, Ar-H), 9.03 (s, 1H, N=CH), 9.45 (s, 1H, CSNH), 9.90 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 182.9 (C=S), 162.5 (C-2), 160.1 (C-3), 158.9 (C-4″), 151.4 (C-8), 147.0 (C-1′), 145.6 (C-5), 143.8 (N=CH), 132.2 (C-4′), 130.4 (C-2″ and C-6″), 126.7 (C-3′ and C-5′), 125.1 (C-1″), 122.3 (C-4), 120.0 (C-6), 118.5 (C-2′ and C-6′), 118.2 (C-7), 116.8 (C-9), 112.5 (C-3″ and C-5″), 75.1 (CH_2), 51.4 (OCH_3), 41.5 ($N(CH_3)_2$). EI-MS *m/z*: 531 (M^+). *Anal*. Calcd for $C_{26}H_{25}N_7O_4S$: C, 58.74; H, 4.74; N, 18.44. Found: C, 58.93; H, 4.72; N, 18.49.

2.7f *1-(4-Hydroxybenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide* (**VIf**):Yield = 76 %; M.p. 235–237 °C. IR (KBr) cm^{-1}: 3431 (OH), 3316 and 3274 (NH), 3052 (Ar-CH), 2955 (CH_3-CH), 1739 (C=O), 1652 (C=N), 1604 (C=C), 1548 and 1345 (NO_2), 1283 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.42 (s, 6H, $N(CH_3)_2$), 4.38 (s, 2H, CH_2 linkage), 5.36 (s, 1H, OH), 7.23–8.36 (m, 11H, Ar-H), 9.29 (s, 1H, N=CH), 9.87 (s, 1H, CSNH), 10.35 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 180.0 (C=S), 163.7 (C-2), 163.2 (C-3), 157.6 (C-4″), 151.9 (C-8), 152.5 (C-1′), 145.4 (C-5), 143.1 (N=CH), 133.3 (C-4′), 130.2 (C-2″ and C-6″), 129.8 (C-3′ and C-5′), 127.0 (C-1″), 126.1 (C-4), 125.8 (C-6), 123.5 (C-2′ and C-6′), 121.2 (C-7), 117.5 (C-9), 115.4 (C-3″ and C-5″), 70.4 (CH_2), 45.6 ($N(CH_3)_2$). EI-MS *m/z*: 517 (M^+). *Anal*. Calcd for $C_{25}H_{23}N_7O_4S$: C, 58.02; H, 4.48; N, 18.94. Found: C, 58.15; H, 4.47; N, 18.90.

2.7g *1-(4-(Dimethylamino)benzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl)thiosemicarbazide* (**VIg**): Yield = 72 %; M.p. 201–204 °C. IR (KBr) cm^{-1}: 3329 and 3262 (NH), 3036 (Ar-CH), 2957 (CH_3-CH), 1720 (C=O), 1661 (C=N), 1617 (C=C), 1514 and 1348 (NO_2), 1250 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.49 (s, 6H, $N(CH_3)_2$), 2.92 (s, 6H, $N(CH_3)_2$), 4.15 (s, 2H, CH_2 linkage), 6.87-8.01 (m, 11H, Ar-H), 8.78 (s, 1H, N=CH), 9.28 (s, 1H, CSNH), 10.07 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 178.2 (C=S), 165.9 (C-2), 163.6 (C-3), 152.6 (C-8),

150.5 (C-4″), 150.3 (C-1′), 143.5 (C-5), 140.1 (N=CH), 133.8 (C-4′), 132.8 (C-2″ and C-6″), 128.0 (C-3′ and C-5′), 127.7 (C-4), 124.2 (C-6), 123.0 (C-1″), 122.1 (C-2′ and C-6′), 121.7 (C-7), 119.0 (C-9), 110.9 (C-3″ and C-5″), 78.7 (CH_2), 40.2 ($N(CH_3)_2$), 39.2 ($N(CH_3)_2$). EI-MS *m/z*: 544 (M^+). *Anal*. Calcd for $C_{27}H_{28}N_8O_3S$: C, 59.54; H, 5.18; N, 20.57. Found: C, 59.71; H, 5.16; N, 20.52.

2.7h *1-(3-Nitrobenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide* (**VIh**): Yield = 70 %; M.p. 296–298 °C. IR (KBr) cm^{-1}: 3372 and 3289 (NH), 3024 (Ar-CH), 2960 (CH_3-CH), 1716 (C=O), 1647 (C=N), 1631 (C=C), 1553 and 1325 (NO_2), 1278 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.26 (s, 6H, $N(CH_3)_2$), 4.40 (s, 2H, CH_2 linkage), 6.92–8.05 (m, 11H, Ar-H), 8.74 (s, 1H, N=CH), 9.53 (s, 1H, CSNH), 9.98 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 182.9 (C=S), 165.2 (C-2), 164.0 (C-3), 150.2 (C-8), 145.8 (C-1′), 146.3 (C-4″), 145.3 (C-5), 142.6 (N=CH), 138.1 (C-4′), 134.1 (C-6″), 133.5 (C-1″), 128.9 (C-5″), 126.4 (C-3′ and C-5′), 124.9 (C-4), 123.9 (C-2″), 123.2 (C-6), 122.7 (C-4″), 121.7 (C-2′ and C-6′), 120.6 (C-7), 119.4 (C-9), 75.0 (CH_2), 42.7 ($N(CH_3)_2$). EI-MS *m/z*: 546 (M^+). *Anal*. Calcd for $C_{25}H_{22}N_8O_5S$: C, 54.94; H, 4.06; N, 20.50. Found: C, 55.11; H, 4.07; N, 20.56.

2.7i *1-(3-Chlorobenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide* (**VIi**): Yield = 73 %; M.p. 227–229 °C. IR (KBr) cm^{-1}: 3367 and 3286 (NH), 3012 (Ar-CH), 2956 (CH_3-CH), 1729 (C=O), 1653 (C=N), 1635 (C=C), 1511 and 1338 (NO_2), 1243 (C=S), 762 (C-Cl). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.34 (s, 6H, $N(CH_3)_2$), 4.39 (s, 2H, CH_2 linkage), 7.21–8.13 (m, 11H, Ar-H), 8.95 (s, 1H, N=CH), 9.52 (s, 1H, CSNH), 9.81 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 179.5 (C=S), 161.7 (C-2), 160.4 (C-3), 154.8 (C-8), 148.0 (C-1′), 142.4 (C-5), 139.9 (N=CH), 135.3 (C-4′), 132.8 (C-1″), 132.3 (C-3″), 131.6 (C-4″), 130.9 (C-5″), 128.1 (C-2″), 127.1 (C-3′ and C-5′), 125.5 (C-6″), 123.6 (C-4), 121.6 (C-6), 120.4 (C-2′ and C-6′), 120.2 (C-7), 118.9 (C-9), 73.4 (CH_2), 44.9 ($N(CH_3)_2$). EI-MS *m/z*: 537 (M^{+2}), 535 (M^+). *Anal*. Calcd for $C_{25}H_{22}ClN_7O_3S$: C, 56.02; H, 4.14; N, 18.29. Found: C, 56.23; H, 4.13; N, 18.25.

2.7j *1-(3-Methylbenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide* (**VIj**): Yield = 77 %; M.p. 253–256 °C. IR (KBr) cm^{-1}: 3350 and 3257 (NH), 3049 (Ar-CH), 2992 (CH_3-CH), 1704 (C=O), 1658 (C=N), 1625 (C=C), 1546 and 1330 (NO_2), 1261 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.41 (s, 6H, $N(CH_3)_2$), 2.69 (s, 3H, CH_3), 4.57 (s, 2H, CH_2 linkage), 7.19–8.14 (m, 11H, Ar-H), 9.06 (s, 1H, N=CH), 9.40 (s, 1H, CSNH), 10.15 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 179.6 (C=S), 162.3 (C-2), 160.8 (C-3), 157.5 (C-8), 148.2 (C-1′), 144.7 (C-5), 141.9 (N=CH), 140.7 (C-3″), 135.4 (C-4′), 134.2 (C-1″), 132.5 (C-4″), 131.6 (C-2″), 130.0 (C-5″), 126.1 (C-3′ and C-5′), 125.8 (C-6″), 122.8 (C-4), 122.0 (C-6), 120.7 (C-2′ and C-6′), 120.3 (C-7), 118.6 (C-9), 76.9 (CH_2), 44.3 ($N(CH_3)_2$), 26.7 (CH_3). EI-MS *m/z*: 515 (M^+). *Anal*. Calcd for $C_{26}H_{25}N_7O_3S$: C, 60.57; H, 4.89; N, 19.02. Found: C, 60.74; H, 4.88; N, 19.05.

2.7k *1-(3-Methoxybenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide* (**VIk**): Yield = 78 %; M.p. 272–275 °C. IR (KBr) cm^{-1}: 3342 and 3295 (NH), 3057 (Ar-CH), 2989 (CH_3-CH), 1702 (C=O), 1646 (C=N), 1608 (C=C), 1523 and 1321 (NO_2), 1275 (C=S), 1047 (C-O-C). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.53 (s, 6H, $N(CH_3)_2$), 3.73 (s, 3H, OCH_3), 4.41 (s, 2H, CH_2 linkage), 7.06–8.45 (m, 11H, Ar-H), 9.09 (s, 1H, N=CH), 9.34 (s, 1H, CSNH), 9.78 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 181.3 (C=S), 163.5 (C-2), 160.1 (C-3), 157.1 (C-3″), 155.7 (C-8), 152.9 (C-1′), 146.2 (C-5), 142.0 (N=CH), 134.8 (C-4′), 131.4 (C-1″), 128.9 (C-5″), 127.4 (C-3′ and C-5′), 125.6 (C-4), 124.5 (C-6), 122.3 (C-2′ and C-6′), 120.9 (C-7), 120.5 (C-6″), 117.3 (C-9), 115.2 (C-4″), 110.7 (C-2″), 72.3 (CH_2), 58.1 (OCH_3), 42.6 ($N(CH_3)_2$). EI-MS *m/z*: 531 (M^+). *Anal*. Calcd for $C_{26}H_{25}N_7O_4S$: C, 58.74; H, 4.74; N, 18.44. Found: C, 58.56; H, 4.75; N, 18.48.

2.7l *1-(3-Hydroxybenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide* (**VIl**): Yield = 71 %; M.p. 289–291 °C. IR (KBr) cm^{-1}: 3425 (OH), 3368 and 3303 (NH), 3031 (Ar-CH), 2961 (CH_3-CH), 1727 (C=O), 1660 (C=N), 1619 (C=C), 1525 and 1312 (NO_2), 1246 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.57 (s, 6H, $N(CH_3)_2$), 4.23 (s, 2H, CH_2 linkage), 5.25 (s, 1H, OH), 7.20–8.32 (m, 11H, Ar-H), 8.65 (s, 1H, N=CH), 9.16 (s, 1H, CSNH), 9.89 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 175.1 (C=S), 168.9 (C-2), 165.3 (C-3), 155.4 (C-3″), 152.7 (C-8), 151.4 (C-1′), 146.0 (C-5), 145.5 (N=CH), 135.8 (C-4′), 133.9 (C-1″), 129.3 (C-5″), 128.6 (C-3′ and C-5′), 125.1 (C-4), 123.7 (C-6), 122.9 (C-2′ and C-6′), 121.4 (C-7), 119.2 (C-3″), 118.3 (C-9), 117.1 (C-4″), 116.5 (C-2″), 74.5 (CH_2), 41.8 ($N(CH_3)_2$). EI-MS *m/z*: 517 (M^+). *Anal*. Calcd for $C_{25}H_{23}N_7O_4S$: C, 58.02; H, 4.48; N, 18.94. Found: C, 57.87; H, 4.50; N, 18.91.

2.7m *1-(3-(Dimethylamino)benzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl)thiosemicarbazide* (**VIm**): Yield = 74 %; M.p. 216–218 °C. IR (KBr) cm^{-1}: 3395 and 3251 (NH), 3023 (Ar-CH), 2974 (CH_3-CH), 1716 (C=O), 1652 (C=N), 1620 (C=C), 1537 and 1345 (NO_2), 1251 (C=S). ^{1}H-NMR ($CDCl_3$, 500 MHz) δ ppm: 2.30 (s, 6H, $N(CH_3)_2$), 3.08 (s, 6H, $N(CH_3)_2$), 4.26 (s, 2H, CH_2 linkage), 7.15–8.28 (m, 11H, Ar-H), 8.82 (s, 1H, N=CH), 9.61 (s, 1H, CSNH), 10.34 (s, 1H, CSNH). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ ppm: 178.4 (C=S), 162.6 (C-2), 161.8 (C-3), 150.2 (C-3″), 157.3 (C-8), 153.1 (C-1′), 148.5 (C-5), 145.7 (N=CH), 138.0 (C-4′), 132.6 (C-1″), 130.9 (C-5″), 126.9 (C-3′ and C-5′), 123.2 (C-4), 122.8 (C-6), 121.1 (C-2′ and C-6′), 120.4 (C-7), 119.7 (C-9), 117.5 (C-6″), 115.0 (C-4″), 109.8 (C-2″), 76.8 (CH_2), 43.5 ($N(CH_3)_2$), 42.9 ($N(CH_3)_2$). EI-MS *m/z*: 544 (M^+). *Anal.* Calcd for $C_{27}H_{28}N_8O_3S$: C, 59.54; H, 5.18; N, 20.57. Found: C, 59.70; H, 5.16; N, 20.51.

2.8 *Biological activities*

Analgesic, anti-inflammatory and ulcerogenic potencies of the title analog were estimated. Significance of all established activities was assessed through ANOVA (One way analysis of variance). Carboxy methyl cellulose (CMC; 1 % suspension) was used as medium to administer test and standard drugs orally, for screening their analgesic and anti-inflammatory potency; whereas, drugs were administered as Tween-80 (10 % v/v) suspension by *i.p.* route for determining their ulcerogenicity. At 25 ± 2 °C in colony cages, the animals were maintained in relative humidity of 45–55 % under a 12 h light and dark cycle. Standard animal feeds were used to feed animals.[27] All animals were acclimatized before one week of studies and are separated into set containing six animals each.

2.8a *Analgesic activity*: Tail flick technique was employed to study the analgesic potency of test derivatives. Random sampling method was used to select either sex of 25–35 g Wistar albino mice.[28,29] Reference diclofenac was utilized for comparison and administered orally at 10 and 20 mg/kg dose. Similarly, title derivatives were also administered orally at 10 and 20 mg/kg dose (two dose levels). Immediately before and 30 min, 1, 2 and 3 h after the treatment, the reaction times was recorded. 10 s was used as cut-off time. Using below mentioned equation the PAA (percent analgesic activity) was calculated.

$$\text{Percent analgesic activity} = \frac{\text{Reaction time (in s) after treatment} - \text{Reaction time (in s)before treatment}}{10 - \text{Reaction time (in s) before treatment}} \times 100$$

2.8b *Anti-inflammatory activity*: By carrageenan-induced paw oedema technique using rats the anti-inflammatory potency of title analog was measured.[30] Reference diclofenac was utilized orally at 10 and 20 mg/kg dose for comparison. Similarly, title compounds were also administered orally at 10 and 20 mg/kg dose (two dose levels). With the help of plethysmograph using mercury displacement method the paw volumes were estimated immediately before and 30 min, 1, 2 and 3 h after injection of carrageenan. According to the below mentioned formula the percent paw oedema inhibition (I) was estimated.

$$I = 100 \times \left(1 - \frac{\text{Mean paw volume of rats in test after carrageenan injection} - \text{Mean paw volume of rats in test before carrageenan injection}}{\text{Mean paw volume of rats in control after carrageenan injection} - \text{Mean paw volume of rats in control before carrageenan injection}}\right)$$

2.8c *Ulcerogenicity*: Earlier reported protocol was used to induce ulcer in rat.[31] 150–200 g weighing Wistar strain of Albino rats of either sex was separated into a range of set containing six rats each. Intraperitoneally Tween-80 (10% v/v suspension) was

administered to control group rats only. 1 group of rat was administered with 200 mg/kg of aspirin once daily through intraperitoneal route for 3 days. In the same route, for 3 days another 1 group was administered with 20 mg/kg of diclofenac once daily as standard. Intraperitoneally 20 mg/kg of title derivatives was administered to the remaining groups of rat. Pylorus was ligated on 4th day using previous reported protocol.[32] Rats were fasted for 36 h before the pylorus ligation procedure. Rats were sacrificed after 4 h of ligation. The stomach was detached and unwrapped alongside with the better curving. Ulcer index was determined using earlier reported method.[33]

2.9 *Antimicrobial activity*

In this study the antimicrobial activities of title compounds were screened by agar streak dilution technique. The antibacterial potency of the title derivatives was screened against 4 Gram "+"ve bacteria (*Bacillus cereus* ATCC 11778, *Staphylococcus epidermidis* ATCC 155, *Staphylococcus aureus* ATCC 9144 and *Micrococcus luteus* ATCC 4698) and 3 Gram "−"ve bacteria (*Pseudomonas aeruginosa* ATCC 2853, *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 11298). The antifungal potencies of the synthesized derivatives were examined against 2 fungi (*Aspergillus fumigatus* ATCC 46645 and *Aspergillus niger* ATCC 9029). In Mueller–Hinton broth at 37 °C, bacterial strains were cultured overnight for antibacterial activity screening. Similarly, in YEPDE agar at 30 °C, the yeast was cultured over night for antifungal activity screening. Nutrient agar was used to suspend the test strains to get 5×10^{-5} cfu/mL final density.

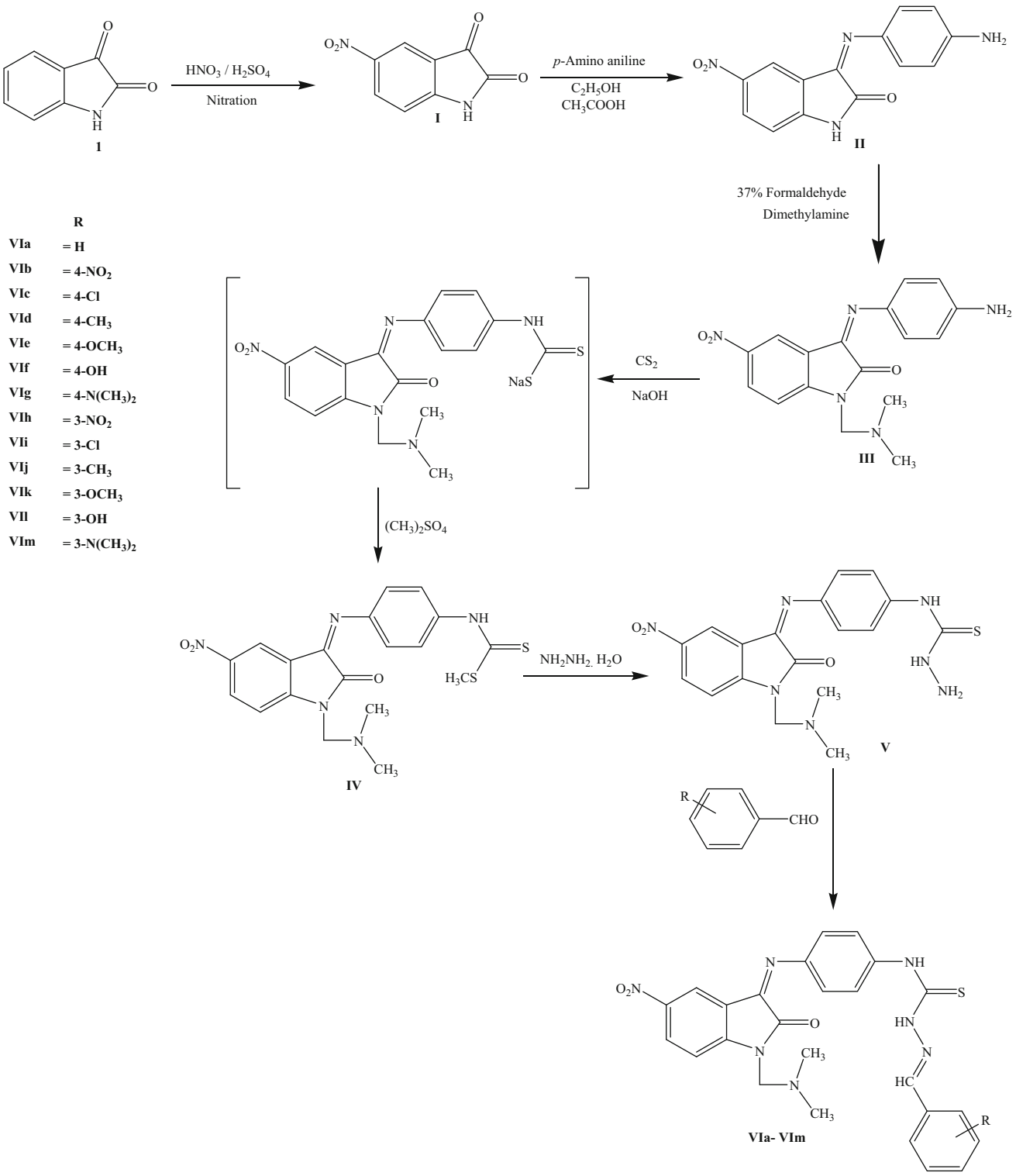
2.9a *Minimum inhibitory concentration (MIC)*: Agar streak dilution technique was used to determine the MIC of the synthesized derivatives.[34] DMF (Dimethyl formamide) was used to prepare 100 µg/mL stock solutions of synthesized derivatives. To specified quantities of molten sterile agar, graded quantities of the synthesized derivative were added. Title compounds incorporated medium (a specified quantity) was poured into a petridish at 40–50 °C to give a 3–4 mm depth and allowed to solidify. Suspension of microorganism was prepared in such a way to have around 5×10^{-5} cfu/mL. Later, microorganism was applied to the petriplates containing successively diluted test derivatives in DMF and incubated for one day and two days at 37 °C for bacteria and fungi, respectively. The lowest concentration of the title derivatives displaying no noticeable growth of bacteria or fungi on the plate was considered as MIC.

3. Results and Discussion

3.1 *Synthesis and characterization*

In current research, a set of novel Mannich and Schiff base of isatin derivatives was synthesized by placing several benzylidene semicarbazide moieties and dimethylaminomethyl group at C-3 and C-1 positions of isatin nucleus, respectively. The title compounds **VIa–VIm** were synthesized as per the protocol displayed in Scheme 1. By a multistep synthesis, various novel 1-(substituted benzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)thiosemicarbazides **VIa–VIm** were synthesized from isatin and *p*-phenylenediamine. Initially, using sulphuric acid and nitric acid, 5-nitroisatin **I** was synthesized from isatin by simple nitration. Schiff base reactions between *p*-phenylenediamine and 5-nitroisatin produced 3-(4-aminophenylimino)-5-nitroindolin-2-one **II** in presence of glacial acetic acid. The synthesized analog 3-(4-aminophenylimino)-5-nitroindolin-2-one **II** undergoes Mannich reaction with dimethylamine (2° amine) and formaldehyde to produce 3-(4-aminophenylimino)-1-((dimethylamino) methyl)-5-nitroindolin-2-one **III**. In the next step, compound **III** reacted with carbon-di-sulphide in presence of NaOH to produce parallel sodiumcarbamodithioate. Subsequently, the obtained sodiumcarbamodithioate was treated with dimethylsulpahte to synthesize methyl 4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl carbamodithioate **IV**. In the pre-final step, compound **IV** reacted with hydrazine hydrate to produce corresponding thiosemicarbazide derivative **V**. Finally, the obtained 4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) thiosemicarbazide **V** underwent Schiff base reaction with various aromatic aldehydes to produce corresponding 1-(substituted-benzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)thiosemicarbazides **VIa–VIm**. Throughout the reactions, TLC was performed to optimize the completion of reactions and its purity.

The projected structure in synthetic scheme was confirmed from elemental and spectroscopic (IR, NMR and Mass) data's of synthesized derivatives. Appearance of peak at 1640 cm^{-1} in IR correlates the existence of C=N moiety in compound **II** which is obtained from *p*-phenylenediamine and 5-nitroisatin **I**. Emergence of singlet at δ 2.59 ppm (dimethyl moiety) and δ 4.17 ppm (CH_2 linkage connects dimethylamine and isatin) confirms the formation of Mannich base derivative **III**. Presence of C=S in



Scheme 1. Synthetic protocols of intermediates and title compounds (**VIa–VIm**).

analog **IV** was established based on IR absorption peak at 1261 cm^{-1} which confirms the formation of compound **IV**. Further NMR spectroscopy confirms this from the appearance of one proton singlet peak at δ 9.16 ppm corresponds to NH and three protons singlet peak for CH_3 at δ 1.88 ppm. The structure of derivative **V** is established from its ^{1}H-NMR spectra by the absence of 3 protons singlet peak of methyl moiety around δ 2.00–2.50 ppm and appearance of two new 1 and 2 protons singlet peaks at δ 2.14 ppm

Table 1. Percent analgesic activity of the synthesized compounds (Tail flick method).

Compound	Dose (mg/kg)	Percent analgesic activity			
		30 min	1 h	2 h	3 h
VIa	10	29 ± 0.94*	34 ± 1.18*	38 ± 0.35*	27 ± 0.72*
	20	38 ± 0.72*	44 ± 1.25*	46 ± 0.43**	35 ± 1.41*
VIb	10	21 ± 1.67**	24 ± 1.41*	29 ± 1.38*	18 ± 1.18*
	20	27 ± 1.50*	31 ± 0.56*	39 ± 1.49*	25 ± 1.32**
VIc	10	43 ± 1.51*	49 ± 0.66**	51 ± 1.73*	36 ± 1.41*
	20	56 ± 1.41*	63 ± 1.87*	73 ± 1.38***	47 ± 1.57**
VId	10	38 ± 1.24**	47 ± 0.66*	51 ± 1.73*	36 ± 1.41*
	20	51 ± 0.73*	60 ± 1.30***	68 ± 1.42**	43 ± 0.84*
VIe	10	18 ± 1.80*	22 ± 1.17*	25 ± 0.51*	18 ± 1.08*
	20	24 ± 1.58*	30 ± 0.75*	32 ± 1.61*	23 ± 2.15*
VIf	10	15 ± 2.17*	19 ± 1.28*	22 ± 1.27*	12 ± 0.96*
	20	20 ± 1.28*	25 ± 1.05**	29 ± 1.18*	18 ± 1.18*
VIg	10	33 ± 0.72*	40 ± 1.15*	44 ± 1.25**	31 ± 1.02**
	20	44 ± 1.09**	53 ± 1.91**	62 ± 0.47*	41 ± 1.08*
VIh	10	20 ± 1.57*	22 ± 1.89*	26 ± 1.14*	17 ± 1.03*
	20	26 ± 0.71*	31 ± 0.59*	36 ± 1.65*	25 ± 0.42*
VIi	10	42 ± 1.56***	47 ± 1.41*	54 ± 0.92**	37 ± 0.75**
	20	54 ± 1.97*	62 ± 0.66*	70 ± 1.19***	46 ± 1.17*
VIj	10	38 ± 1.09*	45 ± 0.75***	51 ± 0.51*	34 ± 0.71*
	20	50 ± 1.81**	58 ± 0.73*	68 ± 1.14**	42 ± 1.65*
VIk	10	18 ± 1.44*	21 ± 1.36*	23 ± 1.80*	16 ± 1.18*
	20	24 ± 0.63*	28 ± 1.51*	31 ± 0.91*	20 ± 1.54**
VIl	10	13 ± 1.46*	18 ± 1.32*	20 ± 1.42*	12 ± 1.58*
	20	19 ± 1.25**	23 ± 0.92*	28 ± 1.36*	15 ± 1.54*
VIm	10	31 ± 1.24*	39 ± 1.91**	41 ± 0.47*	31 ± 1.26*
	20	42 ± 0.47*	50 ± 0.58*	61 ± 1.54**	41 ± 0.91**
Control		3 ± 0.27	5 ± 0.65	8 ± 0.36	2 ± 0.54
Diclofenac	10	39 ± 0.58*	46 ± 0.47***	52 ± 1.55*	35 ± 0.43*
	20	50 ± 0.73**	59 ± 1.62*	68 ± 1.26**	44 ± 1.15*

Each value represents the mean ± SEM (n = 6).

Significance levels *$p < 0.5$; **$p < 0.01$; ***$p < 0.001$ as compared with the respective control.

and 2.40 ppm corresponds to NH of $NHNH_2$ and NH_2 of $NHNH_2$ proton, respectively. Formation of novel 1-(substitutedbenzylidene)-4-(4-(1-((dimethylamino) methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl) thiosemicarbazide **VIa–VIm** from 4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino) phenyl)thiosemicarbazide **V** may established from the disappearance of singlet peak around δ 2.00 ppm corresponds to NH_2 protons. Additionally, in NMR spectroscopy appearance of a variety of other peaks for assigned structure confirms the structure of target derivatives **VIa–VIm**. Molecular weight and purity of synthesized derivatives were confirmed from their mass spectrum.

3.2 *Biological activities*

3.2a *Analgesic activity*: Tail flick method was employed to screen analgesic potency of all target derivatives **VIa–VIm** using Wistar albino mice. Percent analgesic activities of test derivatives are summarized in Table 1. Graded dose response and significant analgesic activity was displayed by nearly all title derivatives. In addition, analgesic data's revealed that synthesized derivatives exhibited reasonable activity at a reaction time of 30 min; at 1st hour the activity increases, at 2nd hour it reaches peak level further and at 3rd hour past its best activities. When compared to standard diclofenac sodium, derivative **VIa** possessing unsubstituted phenyl ring displayed moderate analgesic activity. Increase in activity was observed with dimethylamino derivatives **VIg** and **VIm** due to increased lipophilicity. Further, increase in activity was observed when methyl moiety **VId** and **VIj** replaces dimethylamino group due to further elevated lipid solubility. This activity was found roughly equal to Diclofenac. Further, increase in lipid solubility by exchanging methyl with chlorine

Table 2. Percent anti-inflammatory activity (Carrageenan-induced paw edema test in rats) and ulcer index of the synthesized compounds.

Compound	Dose (mg/kg)	Percent protection				Ulcer index
		30 min	1 h	2 h	3 h	
VIa	10	28 ± 1.27*	30 ± 1.38*	35 ± 1.22*	25 ± 1.64*	ND
	20	33 ± 0.51*	46 ± 0.86*	49 ± 2.37*	30 ± 2.14*	
VIb	10	19 ± 2.24*	24 ± 1.32*	26 ± 1.49*	15 ± 1.20*	ND
	20	25 ± 1.72*	34 ± 0.59*	38 ± 1.05*	24 ± 1.35*	
VIc	10	38 ± 1.14**	44 ± 2.05*	52 ± 2.11**	35 ± 0.91*	0.53 ± 0.25**
	20	48 ± 0.61*	63 ± 0.95***	74 ± 0.56*	46 ± 1.36**	
VId	10	36 ± 2.31*	40 ± 1.34**	47 ± 0.58*	33 ± 1.24*	0.60 ± 0.22*
	20	45 ± 1.18**	57 ± 0.75*	71 ± 1.61**	44 ± 1.39*	
VIe	10	17 ± 1.25*	21 ± 2.05*	23 ± 0.99*	13 ± 0.47*	ND
	20	23 ± 0.40*	30 ± 1.23*	33 ± 1.17*	21 ± 0.95*	
VIf	10	14 ± 0.80*	15 ± 1.15*	20 ± 0.86*	14 ± 1.24*	ND
	20	21 ± 0.82**	26 ± 0.64*	29 ± 2.46*	18 ± 1.97*	
VIg	10	33 ± 1.06*	37 ± 1.38**	45 ± 2.20**	32 ± 0.71*	0.63 ± 0.37*
	20	40 ± 0.71*	53 ± 1.15*	57 ± 0.78*	37 ± 1.39**	
VIh	10	19 ± 1.38*	21 ± 2.17*	25 ± 0.91*	14 ± 2.48*	ND
	20	25 ± 0.97*	31 ± 0.74*	36 ± 2.12*	23 ± 0.81*	
VIi	10	36 ± 1.89**	43 ± 1.55*	51 ± 1.25*	34 ± 0.86***	0.57 ± 0.31**
	20	47 ± 1.62*	61 ± 1.86*	73 ± 2.10**	45 ± 0.53*	
VIj	10	35 ± 0.68**	40 ± 0.51*	47 ± 0.44*	32 ± 0.93*	0.61 ± 0.30*
	20	44 ± 1.34*	57 ± 1.39*	71 ± 1.76**	42 ± 1.57*	
VIk	10	16 ± 0.83*	18 ± 0.50*	21 ± 1.77*	13 ± 1.12*	ND
	20	21 ± 0.95*	28 ± 1.31**	30 ± 1.61*	20 ± 0.59*	
VIl	10	12 ± 1.13*	13 ± 2.12*	17 ± 2.05*	10 ± 0.69*	ND
	20	20 ± 1.60*	25 ± 0.71*	27 ± 0.93*	15 ± 0.78*	
VIm	10	32 ± 0.72**	34 ± 0.33*	41 ± 0.91*	31 ± 0.53**	0.65 ± 0.29*
	20	40 ± 0.74*	51 ± 1.35*	56 ± 0.47**	36 ± 1.49*	
Control		4 ± 0.51	4 ± 0.74	6 ± 0.38	4 ± 0.46	0.16 ± 0.07
Diclofenac	10	35 ± 0.76**	39 ± 2.27*	48 ± 0.47*	32 ± 0.90**	1.59 ± 0.34**
	20	44 ± 0.83*	58 ± 0.45**	71 ± 0.91***	43 ± 1.30*	
Aspirin	200	–	–	–	–	1.83 ± 0.49*

Each value represents the mean ± SEM (n = 6).

Significance levels *p < 0.5; **p < 0.01; ***p < 0.001 as compared with the respective control.

VIc and **VIi** results in reasonable increase in potency, which was found to be more effective than tested standard. A sharp fall in activity was observed, when nitro/methoxy/hydroxy moiety replaces chlorine due to decreased lipophilicity. Compound 1-(4-chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) thiosemicarbazide **VIc** and 1-(3-chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)thiosemicarbazide **VIi** was emerged as potent analgesic agent which is equipotent with tested reference drug.

3.2b *Anti-inflammatory activity*: Anti-inflammatory potency was assessed for synthesized derivatives using Wistar rats by carrageenan-induced paw edema method. Like, analgesic activity entire tested derivatives showed anti-inflammatory potency (Table 2). Title derivatives displayed protection against inflammation induced by carrageenan markedly at 30 min; at 1st hour the potency increases, at 2nd hour it reaches peak level further and at 3rd hour past its best potency. When compared against diclofenac the derivatives having unsubstituted phenyl ring **VIa** displayed only reasonable anti-inflammatory activity. Moderately more activity was displayed by derivatives **VIg** and **VIm** possessing dimethylamino moiety than **VIa** due to increased lipophilicity. Equipotent activity was shown by methyl derivatives **VId** and **VIj** with diclofenac sodium. Amongst all title derivatives, compounds having chloro group **VIc** and **VIi** displayed potent activity which is more than tested standard. When nitro/methoxy/hydroxyl moiety **VIb, VIe, VIf, VIh, VIk** and **VIl** replaces chlorine, a deep

fall in potency was noted. Likewise, analgesic activity compounds **VIc** and **VIi** were emerged as potent analog of the series which is equipotent with tested reference drug.

3.2c *Ulcerogenicity*: Further, more potent title derivatives were screened for their ulcer producing capability and the obtained results are tabulated in Table 2. When compared against reference standard whole screened derivatives displayed less ulcer index. Ulcer index data revealed that, the derivatives possessing chloro substituent **VIc** and **VIi** exhibited less ulcer index, while methyl/dimethylamino moiety substitution results in marginal raise in ulcer index. Among the tested derivatives, 1-(4-chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) thiosemicarbazide **VIc** exhibited least (0.53 ± 0.25) ulcer index which is about 1/3 of reference standards ulcer index.

3.2d *Antimicrobial activity*: Agar streak dilution test is employed to examine the antimicrobial potencies of entire synthesized derivatives. In Table 3, antimicrobial potency of synthesized derivatives was well compared and effectively presented against reference compounds. In parallel experiments, ciprofloxacin and ketoconazole MICs were also estimated with the intention of controlling the sensitivity of the microorganisms. From the antimicrobial data, except **VIb, VIc, VIh and VIi** (MIC: 7.81 μg/mL) entire other derivatives showed lesser activities (MIC: 15.62–62.5 μg/mL) than ciprofloxacin against *S. aureus*. Against *S. epidermidis*, entire series of compounds displayed lower potency (MIC: 15.62–62.5 μg/mL). Compound **VIb** (MIC: 7.81 μg/mL) against *M. luteus* displayed superior potency; derivatives **VIc, VIh and VIi** (MIC: 15.62 μg/mL) showed equivalent potency like reference drug, though rest exhibited minor activity than ciprofloxacin. Against *B. cereus* entire derivatives (MIC: 7.81–125 μg/mL) displayed poorer potencies than ciprofloxacin. Against *E. coli* all the derivatives (MIC: 15.62–125 μg/mL) excluding **VIb** and **VIc** (MIC: 7.81 μg/mL) showed worse activity. Derivatives **VIb** and **VIg** (MIC: 15.62 μg/mL) exhibited equipotency against *P. aeruginosa* like standard. Against *K. pneumoniae* none of the compounds (MIC: 7.81–62.5 μg/mL) displayed identical activity as ciprofloxacin (MIC: 3.9 μg/mL). Entire derivatives (MIC: 15.62–125 μg/mL) against *A. niger* established inferior activity than reference standard. Compound **VIb** (MIC: 15.62 μg/mL) against *A. fumigatus* displayed equipotency though rest of derivatives (MIC: 31.25–125 μg/mL) showed subordinate potency than ketaconazole.

Significant antimicrobial potency was displayed by greater part of the synthesized derivatives. The powerful antibacterial and antifungal potency displayed by derivatives **VIb, VIc, VIh and VIi** may be owing to existence of chloro and nitro moiety (electron-withdrawing group) in structure. Whilst remaining derivatives, though they possess methyl or methoxy or hydroxyl or dimethylamino moiety **VId–VIg** and **VIj–VIm** (electron-donating group) demonstrated less antimicrobial potency (*in vitro*). Moderate antimicrobial activity was displayed by unsubstituted title derivative. The lowest concentration which completely

Table 3. MIC (Minimum inhibitory concentration in μg/mL) of synthesized compounds.

Compounds	*S. aureus*	*S. epidermidis*	*M. luteus*	*B.c ereus*	*E. coli*	*P. aeruginosa*	*K. pneumoniae*	*A. niger*	*A. fumigatus*
VIa	15.62	31.25	62.5	31.25	31.25	31.25	31.25	62.5	62.5
VIb	7.81	15.62	7.81	7.81	7.81	15.62	7.81	15.62	15.62
VIc	7.81	15.62	15.62	7.81	7.81	31.25	7.81	15.62	31.25
VId	15.62	31.25	31.25	7.81	15.62	31.25	15.62	31.25	31.25
Vie	31.25	31.25	62.5	31.25	62.5	31.25	31.25	62.5	62.5
VIf	62.5	31.25	62.5	62.5	62.5	31.25	31.25	62.5	125
VIg	62.5	31.25	125	62.5	62.5	31.25	62.5	125	125
VIh	7.81	15.62	15.62	7.81	15.62	15.62	7.81	15.62	31.25
VIi	7.81	15.62	15.62	7.81	15.62	31.25	7.81	15.62	31.25
VIj	15.62	31.25	62.5	15.62	15.62	31.25	31.25	31.25	62.5
VIk	31.25	31.25	62.5	31.25	62.5	31.25	31.25	62.5	62.5
VIl	62.5	62.5	62.5	125	62.5	31.25	31.25	62.5	125
VIm	62.5	31.25	125	62.5	125	31.25	62.5	125	125
Ciprofloxacin	7.81	7.81	15.62	3.9	7.81	15.62	3.9	–	–
Ketoconazole	–	–	–	–	–	–	–	7.81	15.62

inhibits the observable development of microorganism was considered as MIC. The SARs of the synthesized derivatives exposed that the derivatives having electron accepting groups exhibited superior potency than the derivatives possessing electron releasing moieties. Amongst screened derivatives, 1-(4-nitrobenzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl) thiosemicarbazide **VIb** showed superior potency against *M. leutus*; whilst it exhibited equipotency like reference drug against *P. aeruginosa, E. coli, S. epidermidis* and *A. fumigatus.*

4. Conclusions

In summary, a set of new isatin derivatives was synthesized and characterized using spectroscopic and elemental analyses. Title derivatives i.e., novel isatin Schiff and Mannich bases were screened for their *in vitro* antimicrobial, analgesic, anti-inflammatory and ulcerogenic potencies. Generally, from the study it was found that compounds having chlorine substitution displayed powerful analgesic and anti-inflammatory activities, with small ulcer index. Additionally, it was found that derivatives having electron-withdrawing groups showed superior potency than the derivatives possessing electron-donating moieties. Out of various title derivatives, 1-(4-chlorobenzylidene)-4-(4-(1-((dimethylamino)methyl)-5-nitro-2-oxoindolin-3-ylideneamino)phenyl)thiosemicarbazide **VIc** displayed higher analgesic and anti-inflammatory potency which is greater than diclofenac sodium. Interestingly, compound **VIc** produced only 33% of reference standards ulcer index. Additionally, this derivative also displayed some outstanding antimicrobial potency against *M. leutus, S. epidermidis* and *E. coli*. As a result, this analog might be extended as a novel class of analgesic, anti-inflammatory and antimicrobial drugs. However, additional structural variations are intended to improve these potencies with low ulcer index.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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