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SYNTHESIS OF SOME NEW MANNICH BASES AND BIS(MANNICH BASES) OF PHARMACEUTICAL INTEREST RELATED TO ISATIN SCHIFF BASES

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ABSTRACT

Mannich reaction of isatin Schiff bases **2a-c** with the appropriate *sec*-amine afforded **3a-c**, **4** and the polyhydroxy base **5**. The reaction of **2a-c** with formaldehyde or aromatic aldehyde and the appropriate heteroarylamine gave compounds **6-11**. Treatment of **2b** with glutaric dialdehyde and dimethylamine gave the bis(*N*-Mannich base) **12**. Mannich reaction of **2a-c** with piperazine or TMDP afforded the bis(*N*-Mannich bases) **13a-c** and **14**. The phenolic bis-base **17** was obtained from of the Schiff base **16**. Treatment of **2a, b** with cyclohexanone gave **18a, b** which undergoes Schmidt reaction to give **19a, b**. The periodate oxidation of the tetrahydrocarbazole moiety of **22** provides a convenient access to the generation of the hexahydrobenzo[*b*]azonine system **23**. The newly synthesized compounds were screened for their antioxidant activity and Bleomycine-dependent DNA damage assay. The data showed clearly that compounds **14** and **22b** exhibited the highest antioxidant activities and compounds **14, 17, 19a** and **19b** have an ability to protect DNA from damage induced by bleomycine. Some of the tested compounds gave good activity by bleomycine-dependent DNA damage assay than ABTS antioxidant assay.

Keywords: Mannich bases, Bis(*N*-Mannich bases), Schiff bases, Polyhydroxy bases

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INTRODUCTION

The *N*-Mannich bases derived from isatin (indolin-2,3-dione) and its derivatives have received significant attention due to their wide range of biological and pharmacological activities **[Da Silva et al., (2001), Pandeya et al., (2005), Varma et al., (2009) and Cerchiaro et al., (2006)]**. In particular, much interest has centered around *N*-Mannich bases of isatin, and its Schiff bases, which possess a broad spectrum of action including antibacterial **[Pandeya et al., (2000) (Arzneim. Forsch), Sridhar et al., (2001), Ravichandran et al., (2007) and Chhajed et al., (2010)]**, anticonvulsant **[Sridhar et al., (2002), Varma et al., (2004), Gursoy et al., (1996)]**, anti-HIV **[Pandeya et al., (2000) (Arzneim. Forsch), Sridhar et al., (2001), Pandeya et al., (2000) (Eur. J. Med. Chem.) and Pandeya et al., (2001)]**, antifungal **[Pandeya et al., (2005), Varma et al., (2009), Cerchiaro et al., (2006), Pandeya et al., (2000) (Arzneim. Forsch) and Bal et al., (2005)]**, cytotoxic and anticancer **[Karali et al., (2005), Vine et al., (2009), Solomon et al., (2009)]** activities.

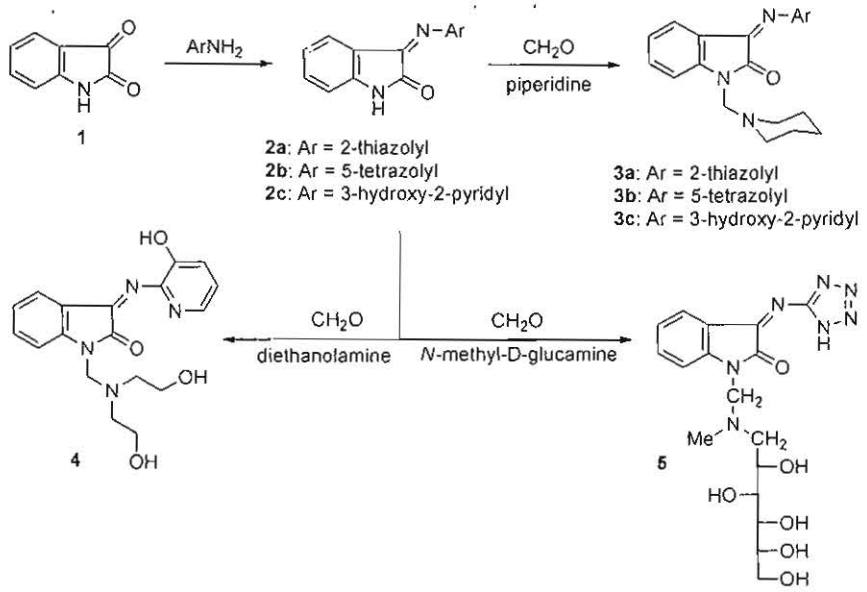
In connection with our studies in the area of Mannich bases **[Afsah et al., (2007), Afsah et al., (2008), Afsah et al., (2011), Hamama et al., (2011), Afsah et al., (2009), Afsah et al., (1984), Afsah et al., (2011) and M. Hammouda et al., (1993)]**, the present work is concerned with attempts to extend the scope of the Mannich reaction with isatin Schiff bases, to including the synthesis of some new *N*-Mannich bases, polyhydroxy bases and bis (*N*-Mannich bases) of potential pharmaceutical applications.

1.RESULTS AND DISCUSSION

1.1.Chemistry

In the present study, isatin (**1**) was treated with 2-aminothiazole, 5-aminotetrazole and 2-amino-3-hydroxypyridine to give the corresponding 3-(heteroarylimino)indolin-2-ones **2a-c**, respectively. Application of Mannich reaction to compounds **2a-c** has been of considerable importance in the synthesis of certain *N*-Mannich bases and bis-bases, which possess considerable synthetic and pharmaceutical interest (**Scheme 1**). Therefore, treatment of **2a-c** with piperidine and

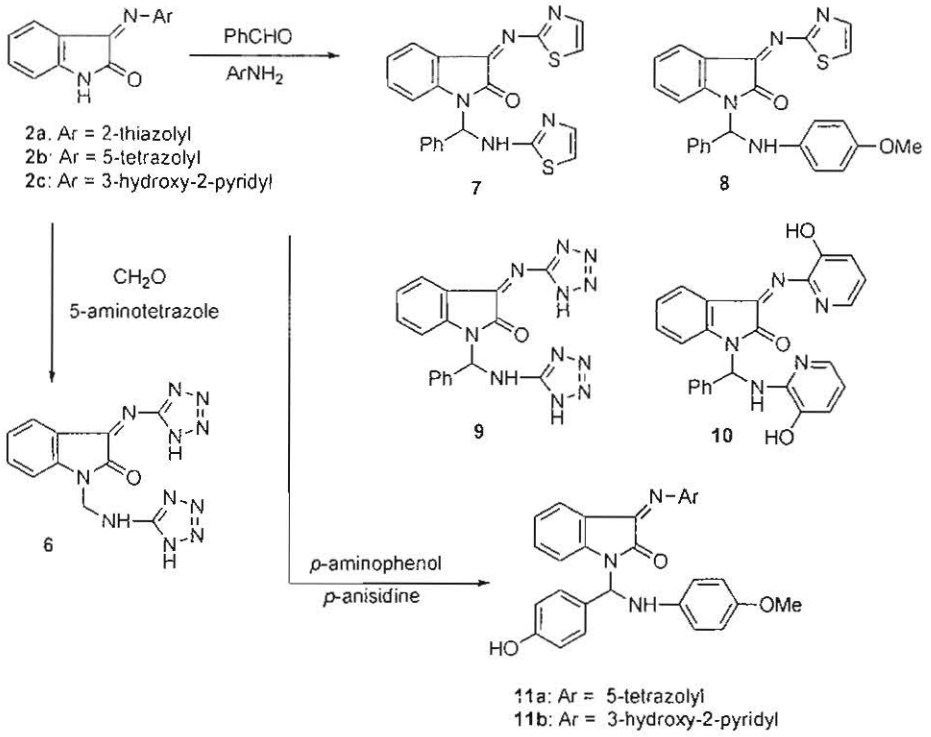
formaldehyde afforded 3-(heteroarylimino)-1-(piperidin-1-ylmethyl) indolin-2-ones **3a-c**, respectively. The analogous reaction of **2c** with diethanolamine gave **4**. One main goal of the present work is to study the possible synthesis of polyhydroxy Mannich bases of the type **5**. This has been realized by treating **2b** with formaldehyde and *N*-methyl-D-glucamine to afford 3-((1*H*-tetrazol-5-yl)imino)-1-((methyl((2*S*,3*R*,4*R*,5*R*)-2,3,4,5,6-pentahydroxy-hexyl)amino)methyl)indolin-2-one (**5**). The analytical and spectral data of compounds **2a-c**, **3a-c**, **4** and **5** are consistent with their structures.



Scheme (1): Synthesis and reactions of 3-(heteroarylimino)indolin-2-ones **2a-c**

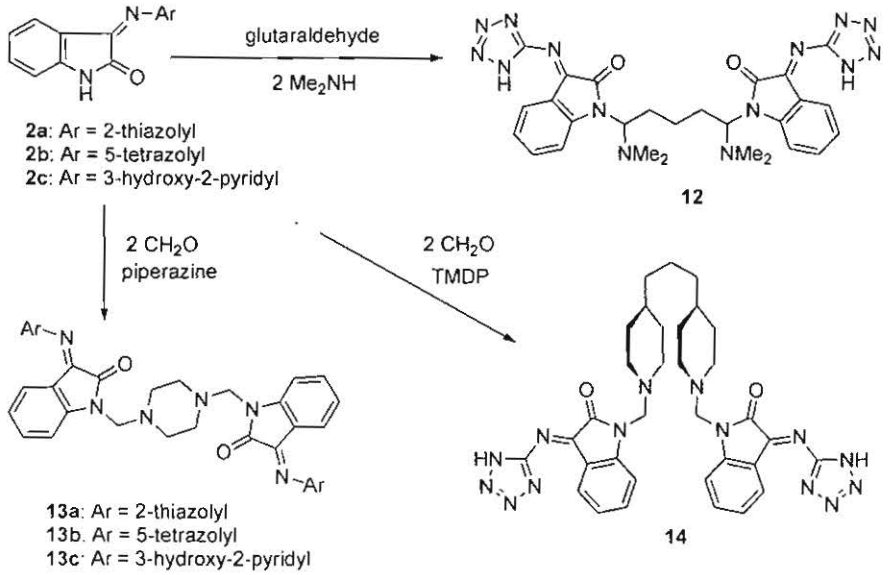
In addition, the use of 5-aminotetrazole in the Mannich reaction with **2b** lead to the formation of 1-(((1*H*-tetrazol-5-yl)amino)methyl)-3-((1*H*-tetrazol-5-yl)imino)indolin-2-one (**6**). The scope of the above synthesis was developed by treating compounds **2a-c** with benzaldehyde and 2-aminothiazole, *p*-anisidine, 5-aminotetrazole and 2-amino-3-hydroxypyridine to afford 1-(heteroaryl-aminobenzyl)-3-(heteroarylimino) indolin-2-ones **7-10**, respectively. A similar reaction takes place on treating **2b** and **2c** with *p*-hydroxybenzaldehyde and *p*-

anisidine yielding compounds **11a** and **11b** (**Scheme 2**). The particular value of this reaction lies in its applicability to a variety of aromatic aldehydes and aromatic or heterocyclic amines, and thus allows considerable variation in the aldehyde and amine components of the aminobenzyl moiety of the products. The structure of **7-10**, **11a** and **11b** was confirmed on the basis of analytical and spectral data.



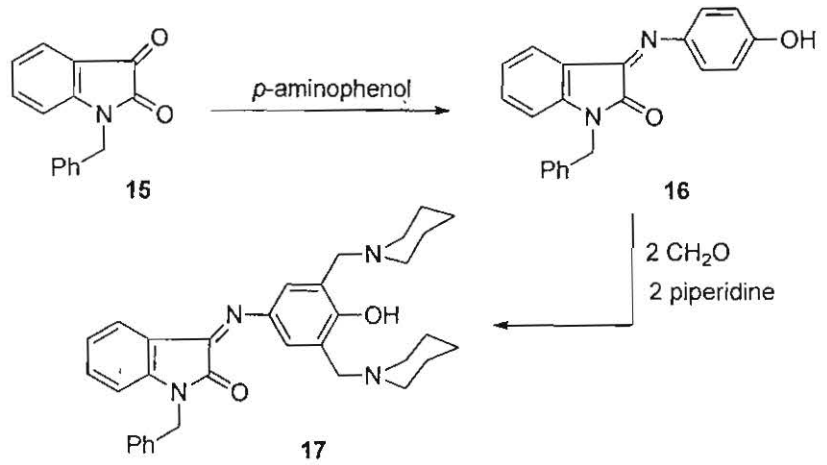
Scheme (2): Reactions of 3-(heteroarylimino)indolin-2-ones **2a-c** with different amines

In the course of this study, the synthesis of the bis(*N*-Mannich base) (**12**) has been achieved by treating **2b** with glutaric dialdehyde and dimethylamine. The use of piperazine in the Mannich reaction with **2a-c** lead to the formation of 1,1'-(piperazine-1,4-diylbis(methylene))bis(3-(heteroarylimino)indolin-2-one) derivatives **13a-c**. The reaction of **2b** with 4,4'-trimethylenedipiperidine (TMDP) and formaldehyde proceeded equally well, providing the bis(*N*-Mannich base) **14** (**Scheme 3**).



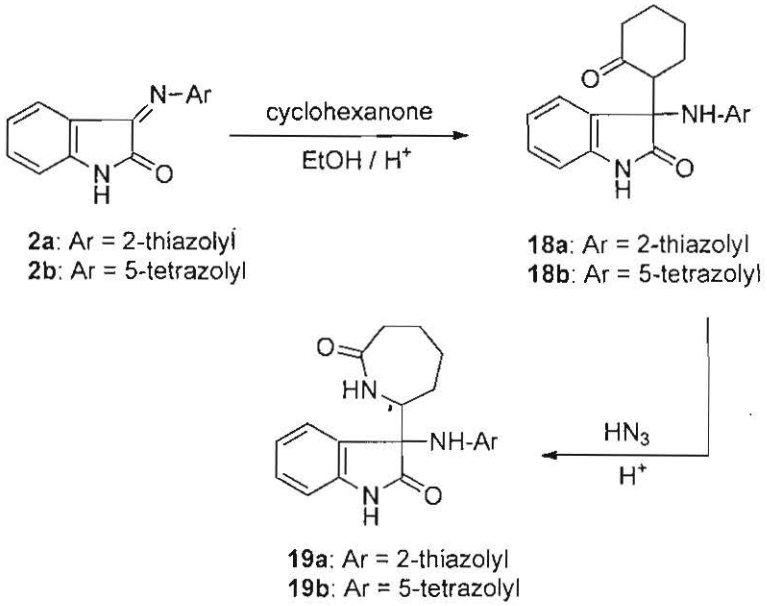
Scheme (3): Synthesis of bis(*N*-Mannich base) (**12**), 1,1'-(piperazine-1,4-diylbis (methylene))bis(3-(heteroarylimino)indolin-2-one) derivatives **13a-c** and bis(*N*-Mannich base) **14**

In addition, Mannich reaction of the phenolic Schiff base **16** is of particular interest, because it provides access to 1-benzylindolin-2-ones having a phenolic Mannich base as a structural unit. This has been achieved by treating 1-benzylindoline-2,3-dione (**15**) **[Aboul-Fadl et al., (2003)]** with *p*-aminophenol to give **16**, which was subjected to Mannich reaction with piperidine and formaldehyde in a molar ratio (1:2:2) to afford 1-benzyl-3-((4-hydroxy-3,5-bis(piperidin-1-ylmethyl)phenyl) imino)indolin-2-one (**Scheme 4**).



Scheme (4): Synthesis of 1-benzyl-3-((4-hydroxy-3,5-bis(piperidin-1-ylmethyl) phenyl)imino)indolin-2-one (**17**)

On the other hand, the reaction of Schiff bases with cycloalkanones has opened routes to the corresponding Mannich bases **[Roth et al., (1970) and Kidwai et al., (2005)]**. Thus, the synthesis of the β-amino ketones (Mannich type bases) **18a** and **b**, incorporating the 2-indolinone moiety, has been achieved by treating compounds **2a** and **b** with cyclohexanone in acidic medium. The Schmidt reaction with **18a** and **b** constitutes an interesting approach towards the synthesis of the ternary heterocyclic systems: 3-(7-oxoazepan-2-yl)-3-(thiazol-2-ylamino)indolin-2-one (**19a**) and the 3-(1*H*-tetrazol-5-yl)amino) analog (**19b**), respectively (**Scheme 5**). The assignment of the (NH) group between the (CO) group and the substituted carbon atom of **19a**, **b** is based on previous studies on the Schmidt rearrangement **[Wolff et al., (1964), Beckwith et al., (1970) and Buehler et al., (1970)]**, and there is much evidence that bulky substituents at the α-position exert stereocontrol on the reaction **[Smith et al., (1948), Smith et al., (1961), Afsah et al., (1984), Afsah et al., (1993) and Hamama et al., (1988)]**.



2a: Ar = 2-thiazolyl
2b: Ar = 5-tetrazolyl

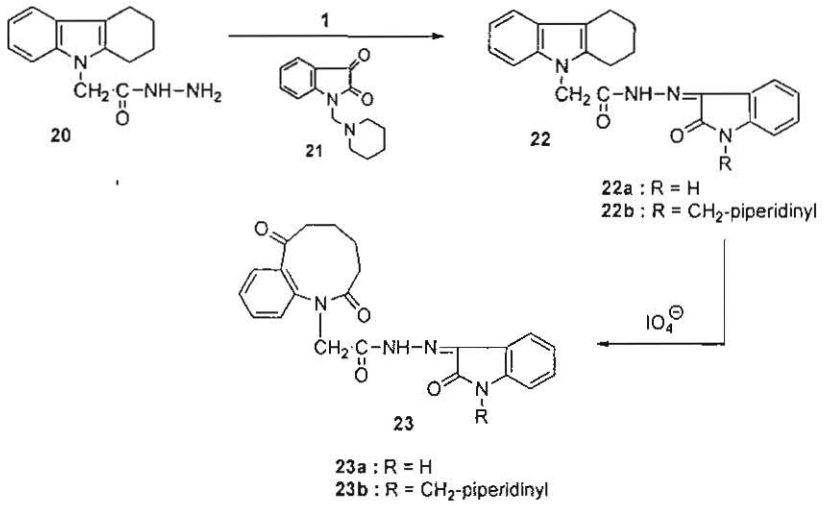
18a: Ar = 2-thiazolyl
18b: Ar = 5-tetrazolyl

19a: Ar = 2-thiazolyl
19b: Ar = 5-tetrazolyl

Scheme (5): Synthesis of 3-((heteroaryl)amino)-3-(7-oxoazepan-2-yl) indolin-2-one derivatives **19a** and **19b**

In an extension of this study, we prepared *N*-(2-oxoindolin-3-ylidene)-2-(5,6,7,8-tetrahydrocarbazol-9-yl)acetohydrazide (**22a**) and its isomer **22b** by treating 1 with 2-(3,4-dihydro-1*H*-carbazol-9(2*H*)-yl)acetohydrazide (**20**) and *N*-piperidinomethyl isatin **21 [Hellmann et al., (2011) and Dolby et al., (1966)]**, respectively, as reported recently by Srinivas **[Srinivas et al., (2011)]**. The periodate oxidation of the tetrahydrocarbazole moiety of **22** is of particular interest, because it provides a convenient access to the generation of the hexahydrobenzo[*b*]azonine system **23** (**Scheme 6**). The formation of **23** is in line with the reported periodate oxidation of tetrahydrocarbazole and related compounds to hexahydrobenzo[*b*]azonines **[Afsah et al., (2009) and Dolby et al., (1966)]**, The synthesis of **23a,b** is of particular interest, because the azonine core is present in the vinblastine and vincristine alkaloids [illegible]

studied with interest centered on their potential pharmaceutical activity as antimalarials **[Klayman et al., (1979)]**, analgesics **[Clark et al., (1988)]**, antihypertensive **[Thorsett et al., (1986)]** and CNS activity **[Elison et al., (1971)]**.



Scheme (6): Synthesis of *N'*-(1-substituted-2-oxoindolin-3-ylidene)-2-(2,7-dioxo-2,3,4,5,6,7-hexahydro-1*H*-benzo[*b*]azonin-1-yl)acetohydrazides **23a** and **23b**

1.2. Biological activity

1.2.1. ABTS Antioxidant assay

The antioxidant activity of the synthesized compounds was evaluated by the method of **[Lissi et al., (1999)]**. The antioxidant activity assay employed here is one of the several assays that depend on measuring the consumption of stable free radicals, i.e. they evaluate the free radical scavenging activity of the investigated component. The methodology assumes that the consumption of the stable free radical (X') will be determined by reactions as follows:

$$XH + Y' \rightarrow X' + YH \qquad (1)$$

The rate and/or the extent of the process measured in terms of the decrease in X' concentration would be related to the ability of the added compounds to trap free radicals. The decrease in color intensity of the free radical solution due to scavenging of the free radical by the

antioxidant material is measured calorimetrically at a specific wavelength. The assay employs the radical cation derived from 2,2'-azino-bis-(3-ethyl benzthizoline-6-sulfonic acid) (ABTS) as stable free radical to assess the antioxidant potential of the investigated compounds.

Some of the isatin derivatives exhibited an antioxidant effect as shown in Table 1. Compared with the control (ascorbic acid), the antioxidant potency of compounds **14** and **22b** were found to be highest, while compounds **18a**, **18b**, **19a**, **19b** and **13** showed good antioxidant activity and the rest of tested compounds showed antioxidant activity. Compounds **14**, **12b** exhibited a high antioxidant activity compared to the new synthesized compounds.

Table (1): Average inhibition (%) of superoxide anion of different extracts/fractions.

Compound No.	ABTS Average % inhibition	Beleomycine - dependent DNA damage (Absorbance)
2a	18.3% ± 1.02	0.151
2b	10.4% ± 1.07	0.111
2c	10.3% ±.05	0.120
3a	11.3% ± 0.06	0.102
3b	17.8% ± 1.12	0.250
3c	16.8% ± 1.12	0.189
4	18.2% ± 0.12	0.104
5	18.4% ± 0.02	0.135
6	12.3% ± 0.06	0.144
7	11.9%* ± 1.14	0.100
8	13.7% ± 1.53	0.099

Cont. Table (1)

9	12.3% ± 1.12	0.097
10	11.3% ± 1.12	0.108
11	16.3% ± 1.01	0.213
12	17.7% ± 0.52	0.183
13	51.28% ± 1.12	0.095 , 0.101
14	61.21% ± 0.12	0.059
18a	54.8% ± 1.23	0.093
18b	54.8% ± 1.23	0.093
19a	51.28% ± 1.12	0.095
19b	51.28% ± 1.12	0.101
22b	61.21% ± 0.12	0.048
23b	11.3% ± 1.13	0.156
Ascorbic acid	78.7% ± 1.02	

*$ABTS^+$ Scavenging activity (%) = [Ac-As/Ac] x 100; Where A_C is the absorbance value of the control and A_S is the absorbance value of the added samples test solution.

Values are means of 3 replicates ± SD, and significant difference at $P < 0.05$ by Student's test.

Bleomycine-dependent DNA damage. -

The compounds of isatin derivatives were also tested for bleomycine dependent DNA damage (Table 1) and showed that compounds **14**, **17**, **19a** and **19b** have an ability to protect DNA from damage induced by bleomycine. Also, compound **14** exhibited a high antioxidant activity compared to the new synthesized compounds. By comparing the results obtained for the antioxidant properties of the compounds reported in this study with their structures, the following structural activity relationship's (SAR's) were postulated: (1)

Compounds **14** and **22b** are the highest potency when compared with ascorbic acid which may be attributable to the presence of piperidine moiety. (2) Compounds **18a, 18b, 19a, 19b** and **13** have good antioxidant activity which may be due to the presence of cyclohexanone, azepine and piperazine moieties.

2. CONCLUSION

Some of the tested compounds gave good activity by bleomycine-dependent DNA damage assay than ABTS antioxidant assay because of the addition of aqueous buffer solution pH 7 reprecipitated the compounds so the antioxidant activity decreased compared to that in case of bleomycine-dependent DNA damage.

3. EXPERIMENTAL

3.1. Instruments

All melting points (uncorrected) were determined on a Gallenkamp electric melting point apparatus. Elemental microanalyses were carried out at Microanalytical Unit, Faculty of Science, Cairo University. Infrared spectra were measured on a Mattson 5000 FTIR spectrometer. The ^{1}H and $^{13}CNMR$ data were obtained in [D_6]DMSO solution on a Varian XL 400 and 100 MHz instrument, respectively, using TMS as internal standard. Chemical shifts are reported in (δ) ppm downfield from internal TMS. Mass spectra were recorded on a GC-MS QP –1000 EX Shimadzu instrument. The course of the reaction and the purity of the synthesized compounds were monitored by TLC using EM science silica gel coated plates with visualization by irradiation with ultraviolet lamp.

3.1.1. Attempted Schiff base with 1: Synthesis of compounds 2a-c

A mixture of isatin **1** (2.9 g, 20 mmol) and 2-aminothiazole (2 g, 20 mmol) or 5-amino-tetrazole (1.7 g, 20 mmol), or 2-amino-3-hydroxy pyridine (2.2 g, 20 mmol) in ethanol (20 mL) and drops of glacial acetic acid (4 drops) was heated on boiling water bath for 30 min. The reaction mixture was allowed to stand at room temperature for 2-10 h. The crystalline products were filtered off to give compounds **2a-c**.

3.1.1.1. 3-(Thiazol-2-ylimino)-indolin-2-one (2a)

Yield (60%); dark red crystals; m.p. 311°C; IR (KBr): ν/cm^{-1}= 3155 (NH), 3049, 3019, 2915 (CH aromatic and aliphatic), 1701 (CO), 1636 (C=N), 1621 (C=C aromatic) 1499, 1333, 824 (C-N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 7.45 - 7.82 (m, 4H, Ar-H), 7.85 – 8.08 (dd, 2H, thiazole protons), 9.23 (br. s, 1H, NH of isatin); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 109 - 137 (6C, aromatic carbons), 145, 152 (CH×2 thiazole), 173 (C=O), 159 (C=N); MS (EI, 70 ev) *m/z* (%) = 229 (50) $[M]^+$, 169 (19), 153 (12), 147 (1), 121 (14), 95 (13), 57 (100). Anal. Calcd. for $C_{11}H_7N_3OS$ (229.25): C 57.6, H 3.08, N 18.33%. Found: C 57.69, H 3.02, N 18.29%.

3.1.1.2. 3-((1H-Tetrazol-5-yl)imino)indolin-2-one (2b)

Yield (65.45%); dark green crystals; m.p. 207 °C; IR (KBr): ν/cm^{-1}= 3455 (NH tetrazole), 3263 (NH isatin), 3074 (CH aromatic and aliphatic), 1732 (CO); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 6.95-7.62 (m, 4H, Ar-H), 9.85 (s, 1H isatin), 11.0 (s, 1H, NH tetrazole); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 119-151 (7C, aromatic carbons), 158 (s, C=N), 184 (s, C=O); MS (EI, 70 ev) *m/z* (%) = 215 (3) $[M+1]^+$, 214 (2) $[M]^+$, 177 (25), 146 (19) [isatin], 120 (41), 137 (4). Anal. Calcd. for $C_9H_6N_6O$ (214.18): C 50.47, H 2.82, N 39.24%. Found: C 50.46, H 2.85, N 39.22%.

3.1.1.3 . 3-((3-Hydroxypyridin-2-yl)imino)indolin-2-one (2c)

Yield (60.54 %); dark red crystals; m.p. 222°C; IR (KBr): ν/cm^{-1}= 3120-3520 (b.s for –OH, NH-isatin), 1720 (CO), 1502 (aromatic ring); MS (EI, 70 ev) *m/z* (%) = 239 (1) $[M]^+$, 146 (30) [isatin], 92 (100), 76 (50). Anal. Calcd. for $C_{13}H_9N_3O_2$ (239.23): C 65.27, H 3.79, N 17.56%. Found: C 65.30, H 3.80, N 17.57%.

3.1.2. Mannich reaction with 2a-c: Synthesis of compounds 3a-c, 4-10, 11a and 11b

General procedure: To a solution of isatin Schiff bases **2a** (1.145 g, 5 mmol), **2b** (1.07 g, 5 mmol), **2c** (1.16 g, 5 mmol) in ethanol (5 mL) was added a mixture of appropriate aldehyde and the desired amine in ethanol (10 mL). The reaction mixture was heated on steam bath for 30 min., then left to stand overnight. The obtained products were filtered off and crystallized from ethanol to give compounds **3a-c**, **4-10**, **11a** and **11b**.

3.1.2.1. 1-(Piperidin-1-ylmethyl)-3-(thiazol-2-ylimino)indolin-2-one (3a)

Yield (44.14%); yellow powder; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 3020, 2932 (CH aromatic and aliphatic), 1720 (CO), 1608 (C=C), 1466, 1037 and 785; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.9 (t, 2H, CH_2-$\underline{CH_2}$-CH_2), 2.12 (t, 4H, CH_2-CH_2), 2.85 (t, 4H, CH_2-N-CH_2 4.23 (N-CH_2-N), 7.92 – 8.24 (dd, 2H, thiazole), 7.16-7.73 (dd, 4H, benzene ring); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 24.12 (-N(CH_2)$_2$-$\underline{CH_2}$-(CH_2)$_2$-N-), 25.72 (2×(N-CH_2-$\underline{CH_2}$-CH_2)), 52.14 (2×(N-CH_2)), 66.23 (N-CH_2-N), 110 - 148 (7C, aromatic carbons), 158.23 (C=N), 178.23 (s, C=O); MS (EI, 70 ev) *m/z* (%) = 326 (17) $[M]^+$, 325 (20) $[M-1]^+$, 242 (56) [M-piperidine unit]$^+$, 228 (100), 92 (52), 77 (45). Anal. Calcd. for $C_{17}H_{18}N_4OS$ (326.41): C 62.55, H 5.56, N 17.17%. Found: C 62.57, H 5.52, N 17.15%.

3.1.1.1. 3-((1H-Tetrazol-5-yl)imino)-1-(piperidin-1-ylmethyl)indolin-2-one (3b)

Yield (68.37%); pale orange powder; m.p. 176°C; IR (KBr): ν/cm^{-1}= 3450 (NH-tetrazole), 3020, 2915 (CH aromatic and aliphatic), 1710 (CO), 1620 (C=C aromatic), 1645 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.3 (t, 2H, -CH_2-$\underline{CH_2}$-CH_2-), 2.40 (t, 4H, $\underline{CH_2}$-N-$\underline{CH_2}$), 3.55 (t, 4H, CH_2-N-CH_2), 4.31(NH), 5.30 (s, 2H, N-CH_2-N), 8.24-8.62 (dd, 4H, Ar-H), 10.23 (s, 1H, NH of tetrazole); MS (EI, 70 ev) *m/z* (%) = 312 (27) $[M+1]^+$, 311 (62) $[M]^+$, 245 (54), 231 (43), 146 (14), 105 (48), 90 (100). Anal. Calcd. for $C_{15}H_{17}N_7O$ (311.34): C 57.87, H 5.50, N 31.49%. Found: C 57.85, H 5.53, N 31.50%.

3.1.1.1. 3-((3-Hydroxypyridin-2-yl)imino)-1-(piperidin-1-ylmethyl)indolin-2-one (3c)

Yield (45.31%); pale brown powder; m.p. 220°C; IR (KBr): ν/cm^{-1}= 3430 (broad band for -OH), 3011, 2914 (CH aromatic and aliphatic), 1713 (CO), 1611 (C=C), 1502 and 486; MS (EI, 70 ev) *m/z* (%) = 336 (4) $[M]^+$, 335 (1) $[M-1]^+$, 252 (56) [M-piperidine unit]$^+$, 238 (50), 92 (100), 77 (50). Anal. Calcd. for $C_{19}H_{20}N_4O_2$ (336.38): C 67.84, H 5.99, N 16.66%. Found: C 67.85, H 5.95, N 16.66%.

3.1.1.1. 1-((Bis(2-hydroxyethyl)amino)methyl)-3-((3-hydroxypyridin-2-yl)imino) indolin-2-one (4)

Yield (45.70%); buff powder; m.p. 189°C; IR (KBr): ν/cm^{-1}= 3210-3570 (broad band, -OH phenolic and alcoholic), 1714(CO), 1606 (C=C), 1504 and 752; MS (EI, 70 ev) *m/z* (%) = 357 (1) $[M+1]^+$, 356 (4) $[M]^+$, 253 (21), 239 (17), 92 (100), 76 (43). Anal. Calcd. for $C_{18}H_{20}N_4O_4$ (356.37): C 60.66, H 5.66, N 15.72%. Found: C 60.64, H 5.63, N 15.76%.

3.1.1.1. 3-((1H-Tetrazol-5-yl)imino)-1-((methyl((2S,3R,4R,5R)-2,3,4,5,6-penta-hydroxy-hexyl)amino)methyl)indolin-2-one (5)

Yield (47.51%); orange crystals; m.p. 165°C; IR (KBr): ν/cm^{-1}= 3520, 3192 (broad band, hydroxyl group), 3022, 2943 (CH aromatic and aliphatic), 1707 (CO), 1650 (C=N), 1590 (C=C), 321, 825, 730 (C-N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.67 (d, 2H, N-$\underline{CH_2}$-CH) , 3.19 (m, 3H, 3CH-OH), 3.41 (d, 2H, CH-$\underline{CH_2}$-OH), 3.62 (t, 1H, N-CH_2-$\underline{CH}$-OH), 4.18 (s, 2H, N-$\underline{CH_2}$-N); 5.12 (br.s, 5H, 5×OH), 7.23 – 7.84 (dd, 4H, Ar-H), 10.19 (s, 1H, NH-tetrazole); MS (EI, 70 ev) *m/z* (%) = 422 (4) $[M+1]^+$, 421 (15) $[M]^+$, 406 (27) $[M\text{-}Me]^+$, 246 (33), 232 (100), 105 (9), 77 (7), 76 (7). Anal. Calcd. for $C_{17}H_{23}N_7O_6$ (421.41): C 48.45, H 5.50, N 23.27%. Found: C 48.41, H 5.52, N 23.30%.

3.1.1.1. 1-(((1H-Tetrazol-5-yl)amino)methyl)-3-((1H-tetrazol-5-yl)imino) indolin-2-one (6)

Yield (58.25%); pale yellow crystals; m.p. 219°C; IR (KBr): ν/cm^{-1}= 3455, 3410 (2NH of 2×tetrazole), 3329 (NH- heteroaryl), 3040, 2950 (CH aromatic and CH aliphatic), 1709 (CO), 1607 (C=C aromatic), 828, 735 (C-N); MS (EI, 70 ev) *m/z* (%) = 312 (2) $[M+1]^+$, 311 (14) $[M]^+$, 226 (19), 187 (27), 147.(100), 93 (57). Anal. Calcd. for $C_{11}H_9N_{11}O$ (311.26): C 42.45, H 2.91, N 49.50%. Found: C 42.43, H 2.93, N 49.54%.

3.1.1.1. 1-(Phenyl(thiazol-2-ylamino)methyl)-3-(thiazol-2-ylimino)indolin-2-one (7)

Yield (45.12%); pale yellow crystals; m.p. 217°C; IR (KBr): ν/cm^{-1}= 3268 (NH), 3049, 3019, 2915 (CH aromatic and aliphatic), 1738(CO), 1655, 1611 (C=N aromatic and C=C), 1499, 824 (C-N); MS (EI, 70 ev) *m/z* (%) = 417 (30) $[M]^+$, 338 (27), 229 (27), 147 (71), 169 (76), 92 (57),

76 (100). Anal. Calcd. for $C_{21}H_{15}N_5OS_2$ (417.51): C 60.41, H 3.62, N 16.77%. Found: C 60.45, H 3.63, N 16.72%.

3.1.1.1. 1-(((4-Methoxyphenyl)amino)(phenyl)methyl)-3-(thiazol-2-ylimino) indolin-2-one (8)

Yield (59.55%); yellow crystals; m.p. 232 °C; IR (KBr): ν/cm^{-1}= 3255 (NH), 3020, 2915 (CH aromatic and aliphatic), 1732(CO), 1502 (aromatic ring); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.61 (s, 3H, -O-CH_3), 8.5 (s, 1H, CH-ph), 7.11 - 7.84 (m, 13H, Ar-H), 7.94 – 8.31 (dd, 2H, thiazole); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 55.80 (-OCH_3), 66.21 (NH-CH-ph), 114.87-157.95 (21C, Ar- carbons), 160.81 (C=N), 198.20 (C=O); MS (EI, 70 ev) *m/z* (%) = 440 (4) $[M]^+$, 364 (12), 272 (59), 229 (32). Anal. Calcd. for $C_{25}H_{20}N_4O_2S$ (440.52): C 68.16, H 4.58, N 12.72%. Found: C 68.24, H 4.52, N 12.76%.

3.1.1.1. 1-(((1H-Tetrazol-5-yl)amino)(phenyl)methyl)-3-((1H-tetrazol-5-yl) imino)indolin-2-one (9)

Yield (52.45%); buff powder; m.p. 232.4°C; IR (KBr): ν/cm^{-1}= 3450, 3192 (2×NH- of tetrazole), 3145 (NH- heteroaryl), 1728 (CO), 1616 (C=N); MS (EI, 70 ev) *m/z* (%) = 389 (1) $[M+2]^+$, 387 (2) $[M]^+$, 347 (7) $[M-Ph]^+$, 247 (5), 232 (45), 147 (50), 92 (100), 76 (50). Anal. Calcd. for $C_{17}H_{13}N_{11}O$ (387.36): C 52.71, H 3.38, N 39.78%. Found: C 52.69, H 3.34, N 39.81%.

3.1.1.1.1-(((3-Hydroxypyridin-2-yl)amino)(phenyl)methyl)-3-((3-hydroxy-pyridin-2-yl)imino)indolin-2-one (10)

Yield (67.42%); dark green crystals; m.p. 237°C; IR (KBr): ν/cm^{-1}= 3421 (broad band, 2×OH group), 3180 (NH), 1714(CO), 1606 (C=C), 1504 and 752; MS (EI, 70 ev) *m/z* (%) = 437 (1) $[M]^+$, 361 (1), 252 (5), 239 (10), 92 (8), 76 (100). Anal. Calcd. for $C_{25}H_{19}N_5O_3$ (437.45): C 68.64, H 4.38, N 16.01%. Found: C 68.68, H 4.34, N 16.05%.

3.1.1.1.3-((1H-Tetrazol-5-yl)imino)-1-((4-hydroxyphenyl)((4-methoxyphenyl) amino) methyl)indolin-2-one (11a)

Yield (62.25%); dark brown crystals; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 3100-3540 (broad band, 2×OH group), 1721 (CO), 1620 (C=N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.72 (s, 3H, -OCH_3), 7.21 – 7.82

(m, 12H, Ar-H), 8.5 (s, 1H, NH-CH-Aryl), 10.2 (br. s, 1H, NH-Aryl), 10.2 (s, 1H, NH- tetrazole); MS (EI, 70 ev) *m/z* (%) = 442 (4) $[M+1]^+$, 441 (2) $[M]^+$, 352 (24) [M-*p*-hydroxyphenyl]$^+$, 247 (4), 232 (36), 147 (23), 92 (106), 76 (35). Anal. Calcd. for $C_{23}H_{19}N_7O_3$ (441.44): C 62.58, H 4.34, N 22.21%. Found: C 62.55, H 4.33, N 22.25%.

3.1.1.1.1-((4-Hydroxyphenyl)((4-methoxyphenyl)amino) methyl)-3-((3-hydroxy-pyridin-2-yl)imino)indolin-2-one (11b)

Yield (42.11%); dark brown powder; m.p. 274°C; IR (KBr): ν/cm^{-1}= 3090-3540 (broad band, 2×OH phenolic), 1702(CO), 1606, 1514 and 754; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 3.68 (s, 3H,-OCH$_3$), 5.81 (s, 1H, -CH-Ph), 7.28-7.92 (m, 15H, Ar-H), 8.46 (s, 1H, NH-Aryl); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 55.79 (-OCH$_3$), 84.71 (CH-ph), 106-145 (23C aromatic carbons), 167.50 (C=N), 184.54 (CO); MS (EI, 70 ev) *m/z* (%) = 467 (1) $[M+1]^+$, 466 (5) $[M]^+$, 364 (7), 253 (14), 239 (2), 147 (14), 91 (100), 76 (15). Anal. Calcd. for $C_{27}H_{22}N_4O_4$ (466.49): C 69.52, H 4.75, N 12.01%. Found: C 69.55, H 4.71, N 12.07%.

3.1.2. Synthesis of 1,1'-(1,5-bis(dimethylamino)pentane-1,5-diyl)bis (3-((1H-tetrazol-5-yl)imino)indolin-2-one) (12)

A mixture of isatine Schiff base (**2b**) (1.07 g, 5 mmol), dimethyl amine (0.45 g, 10 mmol) and glutaraldehyde (0.25 g, 2.5 mmol) in ethanol (20 mL) was heated on steam bath for one hour. The reaction mixture was stirred at room temperature for 24 h and followed by TLC, left to stand for several days to give a gummy material which was solidified by diethyl ether. The dark brown powder that separated was crystalized from ethanol using charcoal and purified by TLC preparative using n-hexane - ethyl acetate (8: 2) to give compound **12**. Yield (58.72%); pale brown crystals; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 3450 (2×NH of tetrazole), 3062, 2959, 2856 (CH aromatic and aliphatic), 1722, 1708 (CO), 1638 (C=N), 1618 (C=C aromatic), 949, 754 (C-N); MS (EI, 70 ev) *m/z* (%) = 583 (17) $[M+1]^+$, 582 (21) $[M]^+$, 508 (25), 344 (17), 265 (25), 231 (22), 146 (25), 108 (13), 81 (20), 76 (100). Anal. Calcd. for $C_{27}H_{30}N_{14}O_2$ (582.62): C 55.66, H 5.19, N 33.66%. Found: C 55.64, H 5.17, N 33.67%.

3.1.3. Bis-Mannich reaction with 2a-c: Synthesis of compounds 13a-c

General procedure: Isatin Schiff base **2a** (1.14 g, 5 mmol) or **2b** (1.079, 5 mmol) or **2c** (1.16 g, 5 mmol) and piperazine (0.22 g, 2.5 mmol) were heated for 30 min. in ethanol (20 mL) containing of formalin 37% (0.15 g, 5 mmol). The reaction mixture was stirred at room temperature for 7 h, then left to stand at room temperature. The obtained products were filtered off and crystallized from ethanol and purified using thin layer chromatography using ethyl acetate - diethyl ether (4:6) as eluent to give compounds **13a-c**, respectively.

3.1.3.1. 1,1'-(Piperazine-1,4-diylbis(methylene))bis(3-(thiazol-2-ylimino)indolin-2-one) (**13a**)

Yield (45.56%); pale orange powder; m.p. > 350 °C; IR (KBr): ν/cm^{-1}= 3022, 2943 (CH aromatic and aliphatic), 1724 (CO), 1640 (C=N), 1597 (C=C), 1496 (C=O); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.74 (br. s., 8H, 4×CH_2 piperazine ring), 4.32 (s, 4H, 2× (N-CH_2-N), 7.23 - 7.72 (m, 8H, benzene ring), 7.90 – 8.23 (dd, 2H, thiazole); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 54 (piperazine ring), 69 (2× (N-$\underline{CH_2}$-N), 110-151 (18C, Ar- carbons), 162.23 (2C=N), 192 (2C=O); MS (EI, 70 ev) *m/z* (%) = 570 (4) $[M+2]^+$, 569 (2) $[M+1]^+$, 568 (3) $[M]^+$, 420 (8), 209 (16), 146 (100). Anal. Calcd. for $C_{28}H_{24}N_8O_2S_2$ (568.67): C 59.14, H 4.25, N 19.70%. Found: C 59.16, H 4.21, N 19.74%.

3.1.3.1. 1,1'-(Piperazine-1,4-diylbis(methylene))bis(3-((1H-tetrazol-5-yl)imino) indolin-2-one) (13b)

Yield (57.13%); dark brown powder; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 3450 (2×NH of tetrazole), 3013-2915 (CH aliphatic and aromatic), 1739 (2CO), 1613 (2C=N), 853; MS (EI, 70 ev) *m/z* (%) = 539 (1) $[M+1]^+$, 538 (10) $[M]^+$, 239 (50), 147 (12), 92 (100), 76 (11). Anal. Calcd. for $C_{24}H_{22}N_{14}O_2$ (538.52): C 53.53, H 4.12, N 36.41%. Found: C 53.50, H 4.09, N 36.43%.

3.1.3.1. 1,1'-(Piperazine-1,4-diylbis(methylene))bis(3-((3-hydroxypyridin-2-yl) imino) indolin-2-one) (13c)

Yield (50.14%); dark green powder; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 3120-3420 (broad band for 2 OH groups), 1709 (2CO), 1612 (C=N), 1468 and 756; MS (EI, 70 ev) *m/z* (%) = 590 (20) $[M+1]^+$, 589 (11) $[M]^+$,

445 (16), 336 (20), 294 (32), 237 (22), 93 (23), 77(100). Anal. Calcd. for $C_{32}H_{28}N_8O_4$ (588.62): C 65.30, H 4.79, N 19.04%. Found: C 65.31, H 4.82, N 19.00%.

3.1.4. Synthesis of 1,1'-((4,4'-(propane-1,3-diyl)bis(piperidine-4,1-diyl)) bis (methylene))bis(3-((1H-tetrazol-5-yl)imino)indolin-2-one) (14)

Isatin Schiff base **2b** (1.07 g, 2.5 mmol) and 4,4`-trimethylene dipiperidine (0.27 g, 1.25 mmol) were heated for 30 min. in ethanol (20 mL) containing formalin 37% (0.1 g, 2.5 mmol). The reaction mixture was stirred at room temperature overnight then left to stand at room temperature. The obtained product was filtered off and crystallized from chloroform to give compound **14**. Yield (37.41%); dark brown powder; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 3449 (2 NH of tetrazole), 1732 (2CO), 1620 (2C=N), 1502 and 852; MS (EI, 70 ev) *m/z* (%) = 663 (23) $[M+1]^+$, 662 (15) $[M]^+$, 460 (28), 376 (50), 334 (19), 239 (32), 147 (14), 92 (100), 76 (45). Anal. Calcd. for $C_{33}H_{38}N_{14}O_2$ (662.75): C 59.80, H 5.78, N 29.59%. Found: C 59.77, H 5.81, N 29.55%.

3.1.5. Synthesis of 1-benzyl-3-((4-hydroxyphenyl)imino)indolin-2-one (16)

A mixture of *N*-benzyl isatin **15** (1.1 g, 5 mmol) and p-hydroxy aniline (0.35 g, 5 mmol) in ethanol (20 mL) and glacial acetic acid (4 drops) was heated on boiling water bath for 30 min. The reaction mixture was allowed to stand at room temperature overnight. The crystalline product was filtered off and purified by TLC preparative using diethyl ether - ethyl acetate (8: 2) to give compound **16**. Yield (52%); pale green crystals; m.p. 264°C; IR (KBr): ν/cm^{-1}= 2650-3250 (broad band OH), 3052, 3020, 2920 (CH aromatic and aliphatic), 1740 (CO), 1660 (C=N), 1620 (C=C),1510, 1340, 840 (C-N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 4.42 (s, 2H, N-$\underline{CH_2}$-Ph), 5.23 (br.s for OH), 6.71-7.68(m, 13H, Ar-H); MS (EI, 70 ev) *m/z* (%) = 328 (34), 236 (240), 146 (100), 121 (14), 95 (14). Anal. Calcd. for $C_{21}H_{16}N_2O_2$: (328.36): C 76.81, H 4.91, N 8.53%. Found: C, 76.83, H 4.87, N 8.57%.

3.1.6. Synthesis of 1-benzyl-3-((4-hydroxy-3,5-bis(piperidin-1-ylmethyl)phenyl) imino)indolin-2-one (17)

A solution of isatin schiff base **16** (1.64 g, 5 mmol) in ethanol (10 mL) was added to a mixture of formaline solution (0.3 g, 10 mmol) and piperidine (0.8 g, 10 mmol) in ethanol (5 mL). The reaction mixture was heated on steam bath for 30 min. then left to stand overnight. The obtained product was filtered off, washed with boiling ethanol (3 × 15 mL) to give compound **17**. Yield (40.15%); dark brown powder; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 2890-3210 (br. OH phenolic), 3030, 2940 (CH aromatic and aliphatic), 1738 (CO), 1610 (C=N), 1456 (C=C), 1040, 785; MS (EI, 70 ev) *m/z* (%) = 523 (12.18), 322 (10.18), 438 (20.42), 354 (36.12), 236 (12.07), 146 (100), 92 (52.17), 77 (45.55). Anal. Calcd. for $C_{33}H_{38}N_4O_2$ (522.68): C 75.83, H 7.33, N 10.72%. Found: C 75.86, H 7.30, N 10.68%.

3.1.7. Acid-catalyzed addition of cyclohexanone with 2a,b: Synthesis of compounds 18a, b

A mixture of Schiff base **2a** (1.14 g, 5 mmol) or **2b** (1.07, 5 mmol) and cyclohexanone (0.49 g, 5 mmol) in ethanol (20 mL) containing 4 drops of concentrated hydrochloric acid was heated under reflux for one hour. The reaction mixture was followed up by TLC during reaction time. The reaction mixture was left to stand at room temperature for several hours. The obtained products were filtered off and crystallized from ethanol and purified by column chromatography using n-hexane - ether (8: 2) to give compound **18a, b**.

3.1.7.1. 3-(2-Oxocyclohexyl)-3-(thiazol-2-ylamino)indolin-2-one (18a)

Yield (81.25%); pale yellow powder; m.p. 194°C; IR (KBr): ν/cm^{-1}= 3322, 3175 (2NH), 3049, 2967 (CH aromatic and aliphatic), 1716, 1697(CO), 1610 (C=C aromatic), 1511, 1469, 832 (C-N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.52 (m, 6H, 3×CH_2), 2.34 (t, 2H, -CO-$\underline{CH_2}$- of cyclohexanone), 3.15 (t, 1H, -CH-CO- of cyclohexanone), 4.43 (s, 1H, NH-heteroaryl), 7.24 - 7.82 (dd, 4H, Ar-H), 7.94 – 8.42 (dd, 2H of thiazole), 9.12 (s, NH of isatine); MS (EI, 70 ev) *m/z* (%) = 328 (1) $[M+1]^+$, 327 (6) $[M]^+$, 230 (16), 229 (100) [M-cyclohexan-one]$^+$, 182 (5). Anal. Calcd. for $C_{17}H_{17}N_3O_2S$ (327.40): C 62.36, H 5.23, N 12.83%. Found: C 62.40, H 5.20, N 12.81%.

3.1.7.1. 3-((1H-Tetrazol-5-yl)amino)-3-(2-oxocyclohexyl)indolin-2-one (18b)

Yield (52.15%); buff powder; m.p. 214°C; IR (KBr): ν/cm^{-1}= 3450, 3322, 3175 (3NH), 3020, 2940 (CH aromatic and aliphatic), 1716, 1695 (2CO), 1610, 1511, 1460 and 840; MS (EI, 70 ev) *m/z* (%) = 313 (11) $[M+1]^+$, 312 (1) $[M]^+$, 233 (14), 232 (100), 147 (41), 77 (50). Anal. Calcd. for $C_{15}H_{16}N_6O_2$ (312.33): C 57.68, H 5.16, N 26.91%. Found: C 57.66, H 5.15, N 26.92%.

3.1.8. Attempted Schmidt reaction with 18a, b: Synthesis of compounds 19a,b

To a solution of **18a** (0.98 g, 3 mmol), **18b** (0.93 g, 3 mmol) in chloroform (10 mL) and concentrated sulphuric acid (3 mL), sodium azide (0.19 g, 3 mmol) was added in small portions during one hour at 0 °C (ice-bath). The reaction mixture was stirred at room temperature for 3 h, then poured on to ice cold water and basified with 40 % ammonium hydroxide. The obtained products were filtered off and crytallized from ethyl acetate, and purified by TLC using chloroform - ethyl acetate (2: 8) to give compounds **19a, b**.

3.1.8.1. 3-(7-Oxoazepan-2-yl)-3-(thiazol-2-ylamino)indolin-2-one (19a)

Yield (65.67%); buff powder; m.p. 226°C; IR (KBr): ν/cm^{-1}= 3410, 3322, 3170 (NH groups), 3054, 2963 (CH aromatic and aliphatic), 1710, 1694(2CO), 1607 (C=C aromatic), 832, 753 (C-N); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.54 – 1.78 (m, 6H, H-3, H-4, H-5), 2.12 – 2.36 (t, 2H, H-6), 4.42 (t, 1H, -CH_2-), 4.54 (s, 1H, NH-hetero aryl), 7.24-6.81 (m, 14H, Ar-H), 7.94 – 8.23 (dd, 2H of thiazol), 8.45 (s, 1H, NH-azepanone), 9.56 (s, 1H, NH of isatine); MS (EI, 70 ev) *m/z* (%) = 343 (27) $[M+1]^+$, 342 (62), 304 (100), 302 (81), 279 (43). Anal. Calcd. for $C_{17}H_{18}N_4O_2S$ (342.42): C 59.63, H 5.30, N 16.36%. Found: C 59.67, H 5.34, N 16.32%.

3.1.9.2. 3-((1H-Tetrazol-5-yl)amino)-3-(7-oxoazepan-2-yl)indolin-2-one (19b)

Yield (58.14%); pale yellow powder; m.p. 244°C; IR (KBr): ν/cm^{-1}= 3450, 3390, 3322, 3170 (NH groups), 3050, 2961 (CH aromatic and

aliphatic), 1720, 1690(2CO), 1607, 842, 753; MS (EI, 70 ev) *m/z* (%) = 328 (11) $[M+1]^+$, 327 (24) $[M]^+$, 239 (81), 147 (12), 92 (100), 76 (50). Anal. Calcd. for $C_{15}H_{17}N_7O_2$ (327.34): C 55.04, H 5.23, N 29.95%. Found: C 54.99, H 5.24, N 28.93%.

3.1.9. Synthesis of N-piperidinomethylisatin (20)

It was prepared according to the reported work [37a].

3.1.10. Synthesis of compounds 22a, b

These compounds were prepared according to the reported work [37b].

3.1.10.1. 2-(3,4-Dihydro-1H-carbazol-9(2H)-yl)-N'-(2-oxo-1-(piperidin-1-yl methyl)indolin-3-ylidene)acetohydrazide (22b)

Yield (52%); pale orange powder; m.p. 242°C; IR (KBr): ν/cm^{-1}= 3340 (NH), 3042, 3015, 2910 (CH aromatic and aliphatic), 1760, 1705 (2 × CO), 1640 (C=N), 1590 (C=C), 870; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.12 – 2.74 (m, 18H, aliphatic protons), 3.83 (s, 2H, N-$\underline{CH_2}$-CO), 4.31 (s, 2H, N-$\underline{CH_2}$-N), (s, 1H, $\underline{NH}$-CO), 7.15 – 7.82 (m, 8H, Ar-H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 25 - 42 (aliphatic carbons), 54 (-CO-$\underline{CH_2}$-N-), 68 (N-$\underline{CH_2}$-N), 96 (C=C), 112 – 145 (12C, Ar-H), 158 (C=N), 182, 194 (2 × CO-N-); MS (EI, 70 ev) *m/z* (%) = 470 (12) $[M+1]^+$, 469 (21) $[M]^+$, 387 (9), 242 (54), 199 (100), 170 (3), 92 (17), 77 (97). Anal. Calcd. for $C_{28}H_{31}N_5O$ (469.58): C 71.62, H 6.65, N 14.91%. Found: C 71.65, H 6.63, N 14.94%.

3.1.11. Synthesis of N'-(1-substituted-2-oxoindolin-3-ylidene)-2-(2,7-dioxo-2,3,4,5,6,7-hexahydro-1H-benzo[b]azonin-1-yl)acetohydrazides 23a and 23b

A solution of **22a** (0.78 g, 2 mmol) or **22b** (0.97 g, 2 mmol) in methanol (40 mL) and acetone (40 mL) was added to a solution of sodium periodate (0.85 g, 4 mmol) in water (5 mL). After stirring at r. t. overnight, the solvent was removed at reduced pressure, and the product was washed successively with water (3× 10 mL) and boiling chloroform (3 × 10 mL) to give **23a** and **23b**, respectively.

3.1.11.1. 2-(2,7-Dioxo-2,3,4,5,6,7-hexahydro-1H-benzo [b]azonin-1-yl)-N'-(2-oxoindolin-3-ylidene)acetohydrazide (23a)

Yield (54%); pale yellow powder; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 3430 (NH- lactam), 3129 (NH- isatin), 3040, 3023, 2920 (CH aromatic and aliphatic), 1770, 1736, 1705 (3 × CO); ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.23 – 1.65 (m, 4H, 3-H_2, 4-H_2), 2.12 – 2.54 (m, 4H, 2-H_2, 5-H_2), 3.89 (s, 2H, N-$\underline{CH_2}$-CO), (s, 1H, $\underline{NH}$-CO), 6.92 – 7.96 (m, 8H, Ar-H), 9.24 (s, 1H, NH- isatin); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 21-52 (aliphatic carbons), 67 (-CO-$\underline{CH_2}$-N-), 105 – 145 (12C, aromatic carbons), 158 (C=N), 164, 172, 181, 192 (4 × CO); MS (EI, 70 ev) *m/z* (%) = 405 (18) $[M+1]^+$, 404 (26) $[M]^+$, 258 (32), 146 (100), 92 (20), 77 (25). Anal. Calcd. for $C_{22}H_{20}N_4O_4$ (404.15): C 65.34 H 4.98, N 13.85%. Found: C 65.36, H 4.95, N 13.81%.

3.1.12.2. 2-(2,7-Dioxo-2,3,4,5,6,7-hexahydro-1H-benzo[b]azonin-1-yl)-N'-(2-oxo-1-(piperidin-1-ylmethyl)indolin-3-ylidene) acetohydrazide (23b)

Yield (42%); buff powder; m.p. > 350°C; IR (KBr): ν/cm^{-1}= 3390 (NH), 3036, 3012, 2912 (CH aromatic and aliphatic), 1765, 1720, 1705 (3 × CO), 1645 (C=N), 1610 (C=C), 872; ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 1.25 – 3.12 (m, 18H, aliphatic hydrogens), 3.82 (s, 2H, N-$\underline{CH_2}$-CO),4.31 (s, 2H, N-$\underline{CH_2}$-N), 5.41 (s, 1H, NH-CO), 7.11 – 7.79 (m, 8H, aromatic protons); ^{13}C-NMR (100 MHz, DMSO-d_6) δ (ppm): 22-42 (aliphatic carbons), 57 (-CO-$\underline{CH_2}$-N-), 70 (N-$\underline{CH_2}$-N), 109 – 151 (aromatic carbons), 162 (C=N), 173, 184, 191 (3 × CON-); MS (EI, 70 ev) *m/z* (%) = 502 (18) $[M+1]^+$, 501 (13) $[M]^+$, 404 (18), 258 (40), 146 (100), 92 (57), 77 (54). Anal. Calcd. for $C_{28}H_{31}N_5O_4$ (501.24): C 67.05, H 6.23, N 13.96%. Found: C 67.08, H 6.25, N 13.92%.

3.2. Pharmacology

3.2.1. Materials and Methods

3.2.1.1. Antioxidant screening; ABTS method [Lissi et al., (1999)]

Antioxidant activity determinations were evaluated from the bleaching of ABTS derived radical cations. The radical cation derived

from ABTS was prepared by reaction of ABTS (60 µl) with MnO_2 (3 mL, 25 mg/mL) in (5 mL) aqueous buffer solution (pH 7). After shaking the solution for a few minutes, it was centrifuged and filtered. The absorbance ($A_{control}$) of the resulting green-blue solution (ABTS radical solution) was recorded at λ_{max} 734 nm. The absorbance (A_{test}) was measured upon the addition of (20µL of 1 mg/mL) solution of the tested sample in spectroscopic grade MeOH/buffer (1:1 v/v) to the ABTS solution. The inhibition ratio (%) was calculated using the following formula:

$$\%\text{Inhibition} = (A_{control} - A_{test}/A_{control}) \times 100 \quad (2)$$

Ascorbic acid (20 µL, 2mM) solution was used as a standard antioxidant (positive control). Blank sample was run using solvent without ABTS (Table 1).

3.2.1.2. Bleomycin-dependent DNA damage

The assay was performed according to Aeschlach et al. and Chan & Tang [Chan et al., (1996)], with minor modifications. L-Ascorbic acid was used as a positive control. The tested compounds were dissolved in DMSO (1 mg/mL). A mixture of DNA (0.5 mg/mL), bleomycin sulfate (0.05 mg/mL), $MgCl_2$ (5 mM), $FeCl_3$ (50 mM) and the sample (20 µL) was prepared. The previous mixture (0.5 mL) was incubated at 37 °C for 1 h, and then the reaction was terminated by addition of 0.05 mL EDTA (0.1 M). The color was developed by adding thiobarbituric acid (TBA) (0.5 mL) (1%, w/v) and HCl (0.5 mL) (25%, v/v) followed by heating at 80 °C for 10 min. After centrifugation, the absorbance of the tested compounds was measured at λ_{max} 532 nm the extent of DNA damage was measured by the increase in absorbance.

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تحضير لبعض قواعد مانش الجديدة و قواعد مانش الثنائية ذات الاهتملم الدوائى ذات الصلة بقواعد شيف لمركب الايزاتين

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تفاعل مانش لقواعد شيف لمركب الايزاتين 2a–c مع بعض الامينات الثنائية المناسبة تعطى 3a–c و 4 و 5. تفاعل المركبات 2a–c مع الفورمالدهيد أو الالدهيد الاروماتى و الامين الاروماتى غير متجانس الحلقة يعطى مركبات 11–6. تفاعل 2b مع جلوتاريك ثنائى الالدهيد و داى ميثيل امين يعطى 12. تفاعل مانش لمركبات 2a–c مع البيبرازين أو TMDP يعطى مركبات 13a–c و 14. تفاعل مانش لقاعدة شيف 16 فى وجود البيبريدين يعطى ثنائى القاعدة 17. تفاعل 2a,b مع السيكلو هيكسانون يعطى مركبات 18a,b التى تتعرض لتفاعل شميدت لكى تنتج مركبات 19a,b. تفاعل أكسدة البيرأيودات لوحدة التيتراهيدروكربازول للمركب 22 يعطى عائلة البنزوأزونين 23. المركبات الجديدة المحضرة تم البحث عن نشاطها كمضادات للاكسدة و مسببات لتلف للحمض النووى المعتمد على البوليميسين. اظهرت النتائج أن المركبات 22b ,14 لها أعلى نشاط مضاد للاكسدة و المركبات 14, 17, 19a, 19b لهم القدرة على حماية الحمض النووى من التلف بسبب البليوميسين. أعطت بعض المركبات نشاط جيد لفحص مسببات لتلف للحمض النووى المعتمد على البوليميسين أكثر من فحص مضادات للاكسدة.