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Original Research Article

FIRST OXIDIZED TETRAINDOLES WITH ANTIMICROBIAL EVALUATION AND STRUCTURE ACTIVITY RELATIONSHIP

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Abstract: : Our novel tetraindoles **1** and **3** that are produced from the acid catalyzed condensation reaction of indoles with aliphatic or aromatic dialdehydes. These tetraindoles have previously synthesized for the first time and published by our group. Tetraindoles **1**, **3** and **5** acts as a good precursor for oxidation or dehydration reactions as well as the well known diindolylmethane afforded the first oxidized tetraindoles **2**, **4** and **6** with good yield. The antimicrobial activity indicated that the tested compounds (aromatic benzene ring between the tetraindole unit) showed inhibitory activities with different degree against all tested pathogenic microorganisms including *B. subtilits, E. coli, C. albicans and Aspergillus niger*.

Keywords: Acid catalyzed condensation reaction, Tetraindoles, Oxidation reaction.

Introduction:

Indole based macrocyclic systems attract increasing interest in a relation to their conformational and self-association properties, stacking interactions, spectroscopic features, cavity shape, and performance as ligands or ion sensing scaffolds.[1] Many indole derivatives have been reported as a primary compound for the synthesis of various drugs and possesses important biological, pharmacological, and medicinal activities.[2-11] Tetraindole products are very similar as two molecules of bis-indolylmethane (BIM) were connected by an alkyl chain $(CH_2)_n$ with n = 1, 2, 3, 4. Cancer chemotherapy with BIMs was recently reviewed and numerous activities are reported.[12] BIMs are found to be very sensitive compounds against the oxidizing agents. The resulting diindolylmethenes were first named "*Rosindoles*" by *Fisher*.[13] The substituted di- or tri- indolylmethenes have been synthesized in literature by the oxidation of the analogous BIMs using oxidizing agents such as DDQ (dichlorodicyanoquinone),[14] TCQ (tetrachloroquinone),[14,15] tritylperchlorate[16] or $FeCl_3$[17] Where if the reaction was accomplished in presence of acid (H^+X^-) as a source of the anion it will afford the

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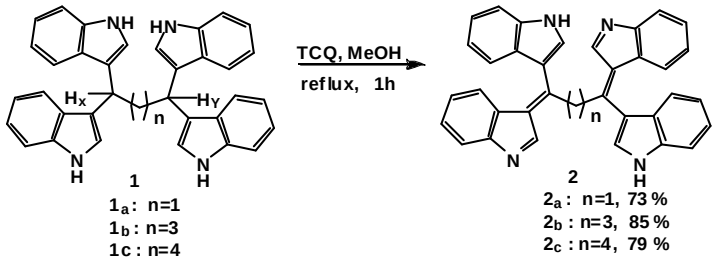
corresponding methylium salts e.g. turbomycin A and turbomycin B.[14, 18] In the absence of the acid the reaction afforded only the free bases compounds.[14] The oxidized form of bis-and tris-indolylmethanes are colour materials utilized as dyes,[14,15,19,20] as well as colorimetric sensors.[20-23]

Oxidation of tetraindoles produced from aliphatic dialdehydes:

Our novel tetraindoles[24] can act as a good precursor for several organic reactions e.g. further condensation reactions[25] and oxidation or dehydration reactions. The stereochemistry of these molecules which illustrated from the Three-dimensional models (3D) for example compounds **1$_a$** (Fig. 2) may leads to expect the highly reactivity of these compounds towards the oxidation chemical reaction where the molecule does not show any stric hindrance of the configuration of the four indole units around the aliphatic hydrocarbon chain. As well as in the oxidation reactions of the diindolylmethanes.[13-23]



Scheme 1: Oxidation reactions of tetraindoles from aliphatic dialdehydes.

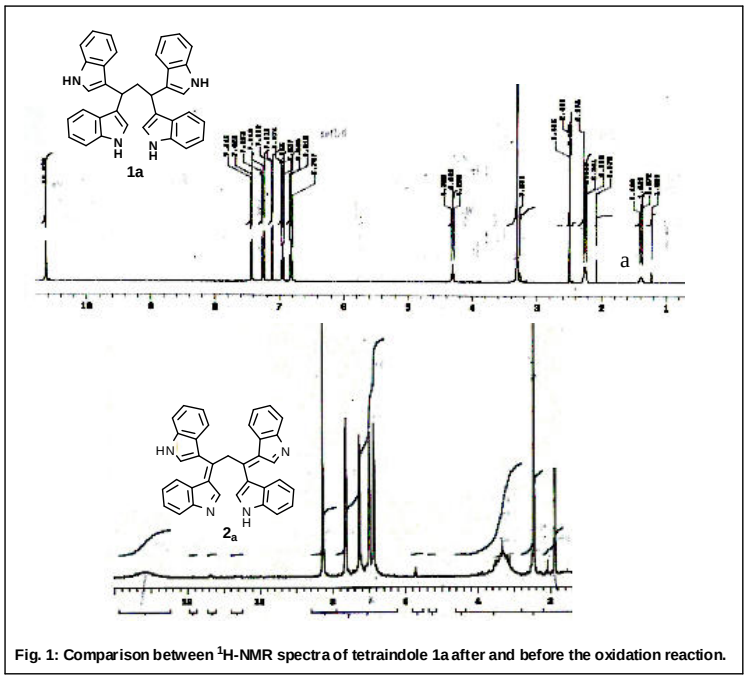


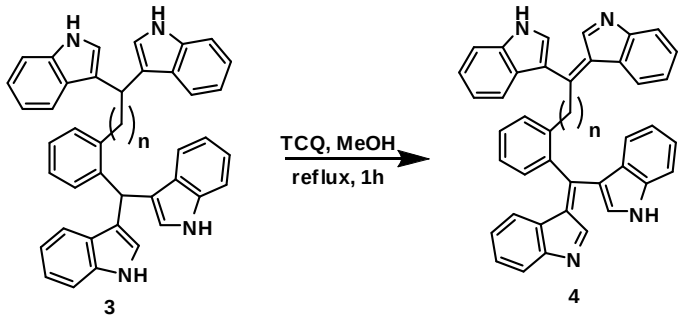
Fig. 1: Comparison between ^{1}H-NMR spectra of tetraindole 1a after and before the oxidation reaction.

The oxidation reaction of tetraindoles (**1$_{a-c}$**) takes place by the same procedure of oxidation of diindolylmethanes. It has been found that these tetraindoles are highly sensitive towards oxidation reagents under similar conditions of oxidation. The reaction was done by using TCQ (tetrachloroquinone) or DDQ (dichlorodicyanoquinone) either in methanol or acetonitrile at room temperature or by reflux for a short time to yield the oxidized form of **2$_{a-c}$** in good yields (73 % - 85 %), (scheme 1).

The products were purified by column chromatography and identified on the bases of their analytical a spectral data. The ^{1}H-NMR spectra of the resulting oxidized forms $\mathbf{2_{a-c}}$ showed the disappearance of the signal related to the protons H_x and H_y in a tetraindoles $\mathbf{1_{a-c}}$. The remaining two acidic protons (NH indole) appear as broad signal at high ppm values. For example the ^{1}H-NMR spectrum of compound $\mathbf{2_a}$, showed a broad signal at δ = 13.17 ppm for the remaining two NH indole protons, (Fig. 1). The broad signal is due to the conjugation within the indoles, where every side of compound $\mathbf{2_a}$ is considered as monoprotonated form of diindolylmethane,[14,26,27] (Fig. 2) shows the conjugated base form of compound $\mathbf{2_a}$ as an example. The conjugated oxidized base forms of type $\mathbf{2_{a-c}}$ are expected to be excellent receptors for the colorimetric detection of anions similar to the oxidized forms of diindolylmethanes. Compounds $\mathbf{2_{a-c}}$ are colour compounds so that they can be used as dyes as well as diindolylmethane,[13-23] These types of compounds showed broad peaks in their IR spectra at 3064 - 3350 cm^{-1}, 3105 - 3340 cm^{-1} and 3250 - 3348 cm^{-1} for the NH indole in compounds $\mathbf{2_a}$, $\mathbf{2_b}$ and $\mathbf{2_c}$ respectively.

Oxidation of tetraindoles produced from *o*-phthalaldehyde homophthalaldehyde and terephthalaldehyde

From the previous oxidation reaction, (scheme 1), which involve the oxidation reaction of the tetraindoles $\mathbf{1_{a-c}}$ yielding bisdehydrated forms $\mathbf{2_{a-c}}$, we found that the tetraindoles which result from the condensation of aromatic dialdehydes (*o*-phthalaldehyde and homophthalaldehyde) compounds $\mathbf{3_{a,b}}$[26] can also undergo dehydration reaction using TCQ affording the dehydrated forms $\mathbf{4_{a,b}}$ in good yields, (Scheme 3).



3a : n=o
3b : n=1

4a : n=0 yield 55 %
4b : n=1 yield 62 %

Scheme 2: Oxidation reaction of tetraindoles 3a,b.

The reaction occurred by similar chemical procedure using TCQ in methanol and reflux under stirring. The colour of the reaction solution turned to deep dark after few minutes. The products were purified by column chromatography eluted with methanol/dichloromethane (5 – 10 %). The ^{1}H-NMR spectra showed no detection for both the aliphatic methylene protons, and detected a broad signal for the remaining two acidic NH indole protons due to the delocalization resulting from the conjugated base. Every side of these oxidized forms appears to be as a monodeprotonated form of oxidized BIMs.[14,26,27] The suggested monodeprotonated forms of **4a** are shown in (Fig. 2). Concerning to the importance of these compounds, we expect them to be favourable receptors for a colorimetric detection,[27] and as a dyes due to their colours as well as the oxidized BIMs.[14-26]

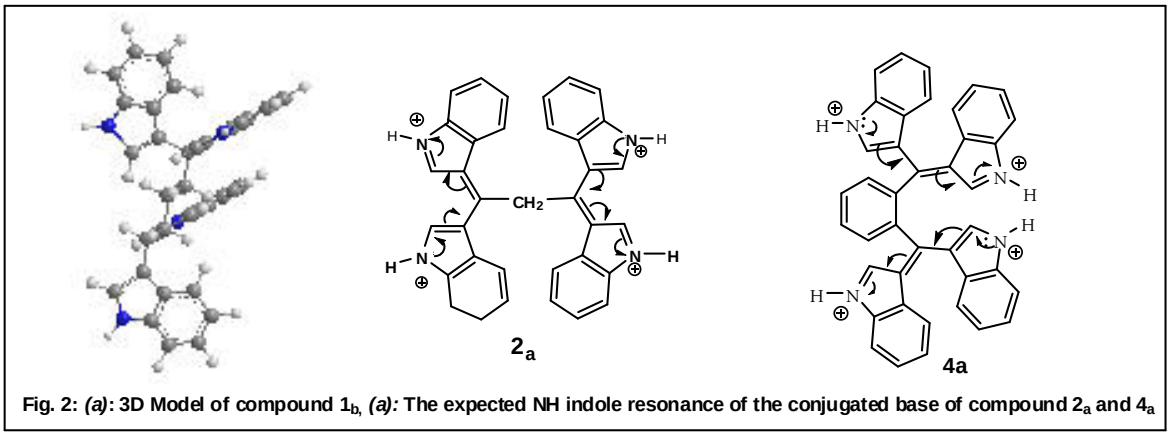
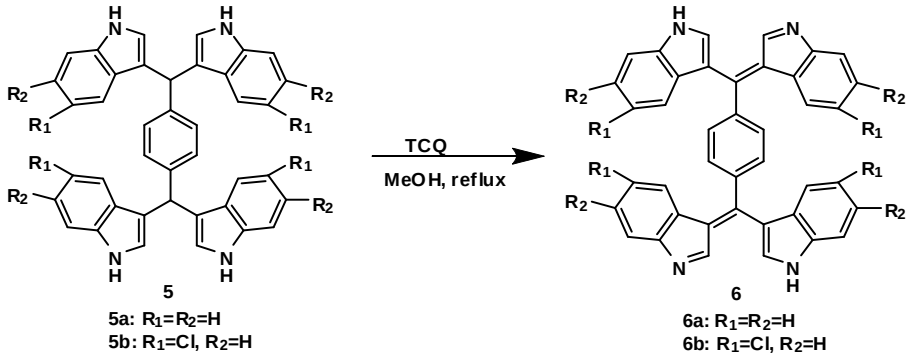


Fig. 2: *(a)*: 3D Model of compound 1_b, *(a)*: The expected NH indole resonance of the conjugated base of compound 2_a and 4_a

The oxidation reaction using TCQ as oxidizing agent in methanol solution has been extended for the synthesis of the expected novel bisdehydrated forms of type **$6_{a,b}$** (Scheme 3). Starting with the well known tetraindole that derived from the condensation reaction of indole with terephthaladehyde.[25,28,29] The reaction occurs as well as the oxidation reaction of all pervious tetrasubstituted indoles which were produced from the condensation of indoles either with aliphatic dialdehydes, compound **1_{a-c}** or aromatic dialdehydes, compounds **$3_{a,b}$**. Compound **$6_{a,b}$** was formed with a high yields, 86% and 90 % with respect to the other oxidized compounds **2_{a-c}** or **$4_{a,b}$**.



Scheme 3: Oxidation reaction of tetraindoles from terephthalaldehyde

The structure of all oxidized tetraindoles 2_{a-c}, $4_{a,b}$ and $6_{a,b}$ has not reported yet and was identified on the bases of their analytical and spectroscopic data. The same as it discussed previously regarding to the expected NH indole resonance structure of 2_{a-c} and $4_{a,b}$ also compounds $6_{a,b}$.

Anti-bacterial activity:

Antimicrobial Results with Structure Activity Relationship

The zone of inhibition of microorganism's growth around the holes and diameters of clear zones were expressed in millimetres as shown in (Table 1). Compounds 1a,b,c showed slightly different biological activity related to the aliphatic carbon chain length. The best one for gram positive bacteria compound 1c and for anti fungi (*C. albicans*) is 1a. The compounds 1a,b,c were inactive against gram negative bacteria. The oxidized products compounds 2a,b,c showed good activity *B. subtilits* and both types of tested fungi compared to compounds 1a,b,c. By inserting the aromatic ring fused to the aliphatic carbon chain compounds 3a,b the activity increased against all tested bacteria and fungi. And hence, **3a,b** showed a moderate inhibitory activity against all tested pathogenic microorganisms except against *C. albicans* showed a strong antifungal activity. The compound **4a** showed a strong inhibitory activity against all tested pathogenic microorganisms except against *A. niger* is

showed a moderate antifungal activity. The compound **4b** is characterized by a moderate inhibitory activity against all tested pathogenic microorganisms The compound **5a** showed a strong antimicrobial activity against *B. subtilis* and *C. albicans*, while showed a moderate antimicrobial activity against both *E. coli* and *A. niger*. As well as, the compound **5b** showed a strong antifungal activity against *C. albicans* and a moderate antibacterial activity against *B. subtilis*, while a weak and negative antimicrobial activity were noticed against *E. coli* and *A. niger*, respectively. The compound **6a** is showed a strong antifungal activity against *C. albicans*, a moderate antimicrobial activity against *E. coli* and a weak inhibitory activity against *B. subtilis* and *A. niger*. The compound **6b** is showed a strong antifungal activity against *C. albicans*, a moderate activity against *B. subtilis* and *E. coli*, while a weak antifungal activity was noticed against *A. niger*. We can conclude that the strongest antimicrobial compound against *B. subtilis* are **4a,4b , 3b** and **5a** in compared to the standard used, while the strongest antimicrobial compound against *E. coli* were **4a,3b** and 5a in comparing with the standard used. On the other hand, we can dissentingly arranged the compounds according to a strong antifungal activity against *C. albicans* as following **4a, 3a,3b, 2a, 6b,6a 5a,5b, 1a** and **2c**. In case of *A. niger* most of compounds varied in the inhibitory activity from moderate to weak, except the compounds **5a**. **3a,2b, 4b** and **4a** were characterized by a moderate antifungal activity.

Conclusion

Our first oxidized tetraindoles **2**, **4** and **6** have been successfully synthesized and identified basically on its analytical and spectral data. The oxidation or dehydration reactions has been done accordingly to the well known diindolylmethane in methanol solution in presence of oxidizing agent affording our first oxidized tetraindoles with high yield. The overall antimicrobial activity denoted that the tested compounds have moderate to strong biological activity against all tested pathogenic microorganisms including *B. subtilits, E. coli, C. albicans and Aspergillus* niger.

Experimental

The melting points were measured on a Boetius-Mikroheiztisch the company "VEB weighing. Rapido Radebeul / VEB NAGEMA "measured and are uncorrected. TLC for the analyzes were with aluminium foil fluorescent indicator from Merck KGaA (silica gel 60 F254, layer thickness 0.2 mm). Rf -values (run level relative to the solvent front). The separations were with column chromatography at atmospheric pressure on silica gel 60 (Grain size from 0.063 to 0.200 mm) from Merck KGaA. NMR spectra were recorded on a "Gemini 2000" (400/100 MHz). The ATR spectra were recorded on a FT-IR spectrometer "IFS 28" by "Bruker. The ESI mass spectra were recorded on a "Finnigan LCQ Classic". The EI mass spectra were recorded on an "Intel 402".

General procedure for the preparation of compounds (2_{a-c}): Compound $\mathbf{1_a}$ or $\mathbf{1_b}$ or $\mathbf{1_c}$ (1 mmol) was dissolved in MeOH (25 ml). TCQ (tetrachloroquinone) (0.34 mg, 1.5 mmol) was added to the reaction mixture. The reaction was allowed to stirring under reflux for 1-1.5 h until the start was finished, when the colour of the reaction became dark red. The product was detected by TLC (5 % MeOH/CH_2Cl_2). Then methanol was evaporated and the product was purified by column chromatography by eluent at first 0.5 L of 100 % CH_2Cl_2, then 0.5 L of 2 % MeOH/CH_2Cl_2 and finally 5 % MeOH/CH_2Cl_2 to afford the pure colord compounds $\mathbf{2_a}$ or $\mathbf{2_b}$ or $\mathbf{2_c}$ respectively

1,3-Di(1*H*-indol-3-yl)-1,3-di(3*H*-indol-3-yl)propane (2_a): dark yellow powder, $C_{35}H_{24}N_4$, 500.59 g/mol, mp: 210 - 214 ^{0}C, ESI-MS: (m/z) = 501.20 [M^++H], IR (ATR, cm^{-1}) = 1455 (CH=N), 2920 (CH_2), 3064 - 3350 (br. NH), ^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.88 (s, 2H, CH_2), 6.88 (d, *J*=7.8 Hz, 4H), 6.99 (t, *J*=7.4, 4H), 7.27 (t, *J*=7.4 Hz, 4H), 7.65 (d, *J*=7.8 Hz, 4H), 8.27 (s, 4H), 13.17 (br., 2H, 2NH), ^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 29.56 (CH_2), 113.95, 119.05, 120.60, 122.74, 124.30, 126.67, 139.61, 140.00, 142.50, 157.74, 171.93(C=N), R_f : 0.03 (10 % MeOH/CH_2Cl_2), yield: 365 mg, 73 %.

No. of samples	Bacteria		F ungi	
	G+ve	G-ve	unicellular	filamentous
	B. subtilits	*E. coli*	*C. albicans*	*Aspergillus niger*
1a	12.12±0.0354	00.00±0.0000	19.98±0.0354	00.00±0.0000
1b	10.98±0.2121	00.00±0.0000	15.44±0.6223	00.00±0.0000
1c	13.15±0.1414	00.00±0.0000	14.99±0.0141	00.00±0.0000
2a	16.90±0.1061	00.00±0.0000	24.88±0.1768	00.00±0.0000
2b	14.08±0.1414	00.00±0.0000	19.98±0.0354	15.13±0.1768
2c	15.10±0.2475	14.93±0.1060	19.90±0.1414	12.13±0.1768
3a	18.18±0.1626	16.98±0.0354	24.98±0.0354	15.16±0.2263
3b	14.12±0.1273	18.13±0.1768	24.98±0.0283	00.00±0.0000
4a	21.09±0.0848	21.98±0.0354	24.99±0.0141	15.11±0.1555
4b	19.25±0.1768	14.98±0.0354	14.98±0.0212	15.13±0.1768
5a	20.88±0.2475	17.99±0.0141	19.98±0.0354	15.18±0.2475
5b	17.18±0.1768	11.88±0.1768	19.88±0.1768	00.00±0.0000
6a	12.88±0.2475	14.98±0.0354	21.98±0.0354	10.63±0.5303
6b	16.18±0.1626	17.13±0.1768	22.99±0.0212	10.68±0.4596
NA*= 30 µg	20.18±0.2475	16.68±0.4596	00.00±0.0000	00.00±0.0000
S* =10µg	14.00±0.0000	00.00±0.0000	12.00±0.0000	00.00±0.0000
TE* =30µg	18.00±0.0000	00.00±0.0000	23.50±2.1213	00.00±0.0000
N* =30 µg	00.00±0.0000	00.00±0.0000	00.00±0.0000	16.00± 0.4142
NS*=100µg	00.00±0.0000	00.00±0.0000	00.00±0.0000	00.00±0.0000
NV*=30µg	29.99±0.0141	30.13±0.1768	00.00±0.0000	00.00±0.0000
T* =30µg	30.63±0.8838	27.98±0.0354	00.00±0.0000	00.00±0.0000
SDZ*=30µg	00.00±0.0000	20.18±0.2475	00.00±0.0000	00.00±0.0000
VA*=30µg	21.99±0.0141	23.18±0.2475	00.00±0.0000	00.00±0.0000

S= Streptomycin; TE = Tetracyciline; N = Neomycin and NS = Nystatin; NA = Negram (nalidixic acid), VA= Vancomycin, CDZ =Cefodizime, NV= Novobiocin, T= Oxytetracycline.

1,5-Di(1*H*-indol-3-yl)-1,5-di(3*H*-indol-3-ylidene)pentane (2_b): dark violet powder, $C_{37}H_{28}N_4$, 528.65 g/mol, mp: > 350 ^{0}C, ESI-MS: (m/z) = 528.47 [M^+], 527.10 [M^+-H], IR (ATR, cm^{-1}) = 1452 (CH=N), 2919 (CH_2), 3105 - 3340 (br, NH), ^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm) = 1.90 - 1.22 (m, 2H, CH_2), 3.41 - 3.44 (m, 4H, $2CH_2$), 6.92 (d, *J*=7.4 Hz, 4H), 7.06 (t, *J*=7.7 Hz, 2H), 7.09 - 7.17 (m, 2H), 7.24 (t, *J*=7.5 Hz, 1H), 7.33 (t, *J*=6.7 Hz, 2H), 7.54 (d, *J*=8.2 Hz, 1H), 7.65 - 7.69 (m, 2H), 7.82 (s, 1H), 7.92 (s, 2H), 8.06 - 8.12 (m, 1H), 8.40 (s, 2H), 11.99 (br., 2H, 2NH), ^{13}C-NMR: (100 MHz, DMSO-d_6) δ(ppm) = 35.00 (CH_2), 40.56

(CH_2), 110.01, 114.24, 121.20, 123.45, 125.50, 127.05, 136.05, 139.55, 140.02, 157.05, 172.42 (C=N), E.A: calcd. C, 84.06; H, 5.34; N, 10.60, found. C, 84.08, H, 5.39, N, 10.62, R_f : 0.05 (7 % MeOH/ CH_2Cl_2), yield: 449 mg, 85 %.

1,6-Di(1*H*-indol-3-yl)-1,6-di(3*H*-indol-3-ylidene)hexane (2_c): dark brown powder, $C_{38}H_{30}N_4$, 542.67 g/mol, mp: 175 - 178 ^{0}C, ESI-MS: (m/z) = 582.40 [M^++K], 541.12 [M^+-H], IR (ATR, cm^{-1}) = 1459 (CH=N), 2852, 2924 (CH_2), 3250 - 3348 (br, NH), ^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 1.42-1.57 (m, 4H, $2CH_2$), 3.23-3.24 (m, 4H, CH_2), 3.41 - 3.57 (m, 2H, CH_2), 6.68 (d, *J*=8.4 Hz, 1H), 6.78 (t, *J*=7.6 Hz, 1H), 6.85 - 6.88 (m, 1H), 6.91 - 7.10 (m, 2H), 7.15 - 7.22 (m, 4H), 7.25 - 7.38 (m, 4H), 7.45 - 7.60 (m, 4H), 7.64 - 7.78 (m, 2H), 7.81 (s, 1H), 9.93 (br., 1H, NH), 10.95 (br., 1H, NH), ^{13}C-NMR: (100 MHz, acetone-d_6) δ (ppm) = 23.69 (CH_2), 43.27 (CH_2), 111.99, 112.99, 118.74, 119.89, 121.71, 123.19, 125.87, 128.68, 137.94, 158.20, 166.50 (C=N), EA: calcd C, 84.10; H, 5.57; N, 10.32, found. C, 84.12, H, 5.61, N, 10.35, R_f: 0.58 (7 % MeOH / CH_2Cl_2), yield: 429 mg, 79 %.

General procedure for the preparation of compound $4_{a,b}$: Compounds 3_a or 3_b (1 mml, 0.6 gm) respectively, was dissolved in MeOH (25 ml). TCQ (tetrachloroquinone) (0.34 gm, 1.5 mmol) was added to the reaction mixture. The reaction was allowed to stir for 2 h under reflux until the reaction was finished, where the colour of the reaction became dark red. The product was monitored by TLC (7 % MeOH/CH_2Cl_2). Then methanol was evaporated and the product was purified by column chromatography by using first 0.5 L of 100 % CH_2Cl_2 then 0.5 L of 2 % MeOH/CH_2Cl_2 and finally 5 % MeOH/CH_2Cl_2 as eluent to afford the pure colored compounds 4_a and 4_b respectively.

1,2-Bis-(1*H*-indol-3-yl)(3*H*-indol-3-ylidene)methyl)benzene (4_a): dark green powder, $C_{40}H_{26}N_4$, 562.66 g/mol, mp: 220 - 223 ^{0}C, ESI-MS: (m/z) = 561.44 [M^+-H], IR (ATR, cm^{-1}) = 1151 (CH=N), 2922 (CH_2), 3253 (NH), ^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 6.91 - 6.99 (m, 4H), 7.05 (d, 2H, *J*=7.8 Hz), 7.11 (t, 2H, *J*=7.6 Hz), 7.20 (t, 2H, *J*=11 Hz), 7.27 - 7.39 (m, 4H), 7.40 (s, 2H), 7.60 - 7.80 (m, 4H), 8.10 (m, 2H), 8.19 (s, 2H), 11.14 (s, br., 2H, 2NH), R_f : 0.1 (7 % MeOH/CH_2Cl_2), yield: 309 mg, 55 %.

3-(2-(2-(1*H*-indol-3-yl)(3*H*-indol-3-ylidene)methyl)phenyl)-1-(1*H*-indol-3-yl)ethylidene)-3*H*-indole (4_b): dark violet powder, $C_{41}H_{28}N_4$, 576.69 g/mol, mp: 150 - 155 ^{0}C, ESI-MS: (m/z) = 576.50 [M^+], 575.49 [M^+-H], IR (ATR, cm^{-1}) = 1067 (CH=N), 2922 (CH_2), 3303 (NH), ^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 3.60 (s, 2H, CH_2), 6.96 - 6.99 (m, 4H), 7.03 (d, 2H, *J*=9.8 Hz), 7.05 (d, 2H, *J*=7.8 Hz), 7.21 (t, 2H, *J*=7.4 Hz), 7.26 - 7.39 (m, 4H), 7.45 (s, 2H), 7.47 - 7.48 (m, 2H), 7.60 - 7.79 (m, 2H), 8.09 (d, 2H, *J*=8.2 Hz), 8.19 (s, 2H), 11.19 (s, br., 2H, 2NH), ^{13}C-NMR: (100 MHz, Acetone-d_6) δ (ppm) = 36.49 (CH_2), 102.45, 112.09, 112.25, 114.34, 119.52, 119.71, 120.09, 120.39, 120.53, 120.84, 121.79, 122.04, 122.32, 122.79, 123.07, 123.27, 123.56, 124.04, 125.39, 125.94, 126.14, 126.35, 126.50, 126.98, 127.19, 127.73, 127.99, 128.47, 128.59, 128.81, 129.79, 131.92, 132.29, 132.39, 137.16, 142.27, 143.95, 148.18, 161.00 (C=N), 162.04 (C=N), R_f : 0.83 (7 % MeOH/CH_2Cl_2), yield: 558 mg, 62 %.

General procedure for the preparation of compound $6_{a,b}$: Compounds 5_a or 5_b (1 mmol) was dissolved in MeOH 25 ml, TCQ (tetrachloroquinone) (0.34 g, 1.5 mmol) was added to the reaction mixture. The reaction was allowed to stir for 2 h under reflux until it was finished. The colour of the reaction mixture became dark red. The product was detected by TLC 5 % MeOH/CH_2Cl_2. Methanol was evaporated and the product was purified by column chromatography by eluting with 1 L of 2 % MeOH/CH_2Cl_2 and then with 5 % MeOH/CH_2Cl_2 to afford the pure colord compounds 6_a or 6_b respectively.

1,4-(1*H*-indol-3-yl)(3*H*-indol-3-ylidene)methyl)benzene (6_a): dark red powder, $C_{40}H_{26}N_4$, 562.66 g/mol, mp: > 350 ^{0}C, ESI-MS: (m/z) = 563.26 [M^++H], 561.35 [M^+-H], IR (ATR, cm^{-1}) = 1459 (CH=N), 3035 – 3450 (br. NH), ^{1}H-NMR: (400 MHz, DMSO-d_6) δ (ppm)

= 6.95 (d, 4H, J=7.9 Hz), 7.05 (t, 4H, *J*=7.5 Hz), 7.27 (t, 4H, *J*=7.6 Hz), 7.59 (d, 4H, *J*=7.8 Hz), 7.69 (s, 4H), 8.23 (s, 4H), 10.91 (s,br., 2H, 2NH), ^{13}C-NMR: (100 MHz, DMSO-d_6) δ (ppm) = 110.92, 111.60, 115.00, 115.94, 116.60, 117.32, 120.68, 120.87, 121.50, 122.96, 125.21, 127.63, 131.82, 134.33, 137.03, 139.34, 140.00, 141.33, 144.04, 161.05 (C=N), R_f-Value: 0.52 (10 % MeOH/CH_2Cl_2), yield: (484 mg), 86 %.

1,4-Bis((5-chloro-1*H*-indol-3-yl)(5-chloro-3*H*-indol-3-ylidene)methyl)benzene (6_b): dark red powder, $C_{40}H_{22}C_{l4}N_4$, 700.44 g/mol, mp: 220 - 225 ^{0}C, ESI-MS: (m/z) = 701.10 [M^++H], 699.27 [M^+-H], IR (ATR, cm^{-1}) = 1130 (CH=N), 3106 – 3450 (br. NH), ^{1}H-NMR: (400 MHz, acetone-d_6) δ (ppm) = 6.64 (s, 2H), 6.87 (s, 2H), 6.97 (dd, 2H, *J*=2.3, 8.6 Hz), 7.12 (d, 2H, *J*=7.9 Hz), 7.29 - 7.34 (m, 4H), 7.42 (d, 2H, *J*=7.8 Hz), 7.47 -7.53 (m, 4H), 8.09 (s, 2H), 10.26 (s,br, 2H, 2NH), EA: calcd. C, 68.59; H, 3.17; Cl, 20.25; N, 8.00, found C, 68.61, H, 3.20, Cl, 20.19, N, 7.98, R_f : 0.33 (7 % MeOH/CH_2Cl_2), yield: 630 mg, 90 %.

Antimicrobial screening:

Samples Preparation

All samples were dissolved in dimethyl sulfoxide (RFCL Limited, New Delhi, India) DMSO at 10 mg/mL concentration as shown in the Table (1) in comparing with different standard antibiotics. Antibiotic discs of Streptomycin (S) (10 µg) , Vancomycin, (VA) (30 µg), Cefodizime), (CDZ) (30 µg), Novobiocin, NV (30 µg), Oxytetracycline (T) (30 µg) and Tetracyciline (TE) (30 µg) were used as positive control for bacteria, (Neomycin (N) (30 µg), Negram (NA nalidixic acid), and Nystatin (NY) (100 µg) were used for fungi and sterilized paper discs without compounds or antibiotics were used as negative controls for both the bacteria and fungi. The experiment was performed in triplicate. The ability to inhibit the growth of Gram-positive and Gram-negative bacteria, yeasts and filamentous fungi was observed using an overlay method [30].

Strains used

The common pathogenic and food spoilage microorganisms were selected for their relevance in bakery products and other food: the gram-positive bacteria; *Bacillus subtilits and Staphylococcus aureaus* and the gram negative bacteria; *Escherichia coli*, yeasts such as *Candida albicans* and fungi *(Aspergillus niger).*

Media used

The bacteria were slanted on nutrient agar (Merck, Darmstadt, Germany), Yeast was slanted and mentioned on Sabaroud's agar medium (Lab M., Bury, Lancashire, UK) and the fungi was slanted and mentioned on the potato Dextrose Agar medium (Lab M Limited, Bury, Lancashire, UK). Mueller-Hinton agar (Lab M., Bury, Lancashire, UK) following the manufacturer's instructions was used for the assay.

Bioassay

The antibacterial screening was essentially by the well diffusion agar method described by *Moosdeen et al.*, (1988) [31]. The organisms were streaked in radial patterns on the agar plates. Plates were incubated under aerobic conditions at 37°C and 28 °C for 24 h and 48 h for bacteria and fungi, respectively. In order to obtain comparable results, all prepared solutions were treated under the same conditions under the same incubated plates. All tests were performed for three replicates. Plates were examined for evidence of antimicrobial activities, represented by a zone of inhibition of microorganism's growth around the holes and diameters of clear zones were expressed in millimetres [32].

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