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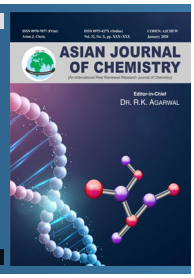

Design, Synthesis, Characterization and Biological Activities of Recent Isatin Derivatives with Proven Pharmacophoric Moiety

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Isatin derivatives, alkylated with proven pharmacophoric moiety were synthesized for various biodynamic activities. To enhance some biodynamic properties like antibacterial and antifungal, imine formation with various compounds at C_3 was analyzed. The isatin derivatives were characterized and examined for various biodynamic activities on bacteria, fungus and cancer cell lines.

Keywords: Isatin, Schiff base, Biological activity.

INTRODUCTION

Derivatives of isatin (1*H*-indol-2,3-dione) possess a versatile bioactivity [1] and used as starting compound for synthesizing a wide range of heterocyclic compounds in drugs syntheses [2-6]. The past studies on isatin derivatives are found to possess antitumor, antibacterial, antifungal, anti-HIV, anti-convulsant, antiviral, anti-inflammatory and other biodynamic properties [6]. Drugs containing the isatin moiety are used to treat diseases such as epilepsy [7], tuberculosis [8] and bulimia [9]. Considering the precedential biological properties of isatin and its derivatives, there is further scope to create and explore isatin derivatives for emerging drug-targets.

Previous research on isatin derivatives shows that mono-substituted isatin at aryl ring has greater cytotoxic and other biodynamic properties over unsubstituted isatin. Further, bromo-substituted isatin was found to possess greater cytotoxic properties over chloro, nitro, hydorxy substituted Isatin. It was found that halogenation substituted isatin derivatives are the most active compounds with 5-bromo, 5-iodo and 5-fluoro isatin being almost 10 times more active than the unsubstituted isatin [10]. Moreover, isatin derivatives with postion 5-substituted were more active than isatin substituted at other positions and found to possess greater anticancer activity [11]. Thus, the starting material for creating prospective isatin derivatives was taken as 5-bromo isatin.

New substances based on proven satin scaffolds in combination with other pharmacophoric elements of drugs can be a right approach for the synthesis of new isatin derivatives for prospective drugs. *N*-Alkylated isatin derivatives are found to have anticancer activities [12-14] and *N*-methylation greatly improved the cytotoxicity of isatin [10,11], SAR studies also showed that an aromatic ring with a one or three carbon atom at position 1-N increased the anticancer activities [15-19]. Thus, considering the above facts and proven pharmacophoric properties of moiety such as ethyl pyrrolidine, ethyl piperidine dimethylamino ethane and diethylamino ethane, intermediate compound *N*-alkylated 5-bromo isatin was synthesized and utilized to design new isatin derivatives with these moieties to investigate for various biodynamic activities such as antitumor, antibacterial and antifungal activities.

Further, previous studies also reported that isatin Schiff bases (imines and hydazones) also increases the anticancer activity on various cell lines [20-23] and also possessed antibacterial activity against *Bacillus subtilis* [24,25] and *Magnaporthe grisea* [26]. Thus, a new set of isatin derivatives by imine formation at C_3 of alkylated compounds were synthesized and screened for hypothesized biodynamic activities such as antitumor, antifungal and antibacterial.

EXPERIMENTAL

The melting points were measured in open capillaries using Toshniwal melting point apparatus and are uncorrected. The

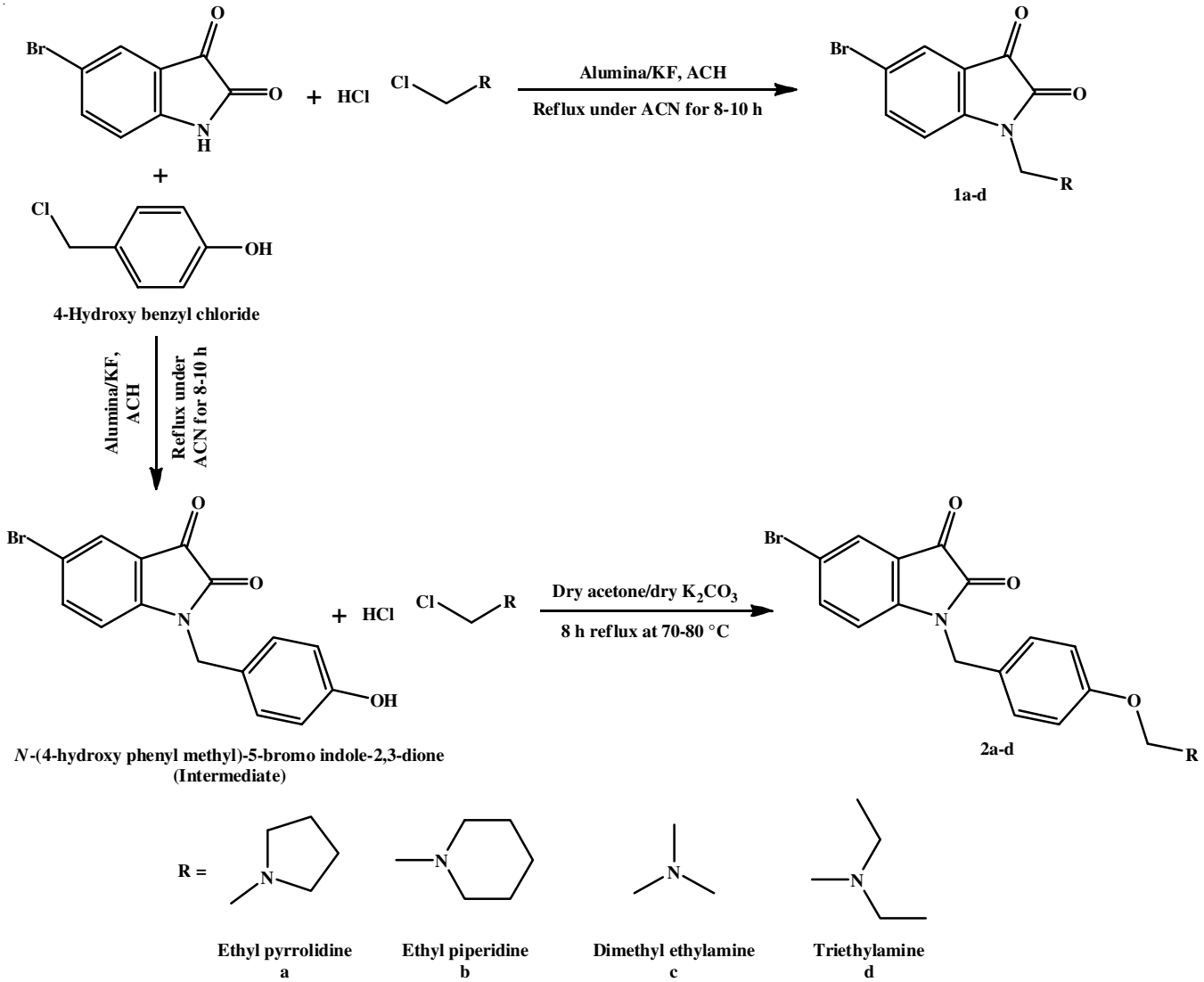
Bruker-Avance DRX 300 (300 MHz, FT NMR) spectrometer was used for recording ^{1}H NMR. Elemental analysis were done on analyzer EA-1108 and the values were within ±3-4% of theoretical values. For checking the purity of products, pre-coated silica gel 60 F_{254} was used and the spots were visualized by using iodine vapours.

General procedure for synthesis of compounds: The prospective compounds were synthesized using base compound 5-bromo isatin (**1**). *N*-(4-Hydroxyphenyl methyl)-5-bromo indol-2,3-dione (**2**) was then synthesized after the alkylation of 5-bromo isatin (**1**) with *p*-hydroxy benzyl chloride.

A set of compounds (**2a-d**) were synthesized by the etherification of compound **2** with pyrrolidylethyl chloride, 2-(piperidyl)ethyl chloride, dimethylaminoethyl chloride, dimethylamino ethyl chloride at *para*-position, respectively. The other set of compounds (**3a-d**) and (**4a-d**) were synthesized by formation of imines at C_3 position of compounds (**1a-d**) and (**2a-d**), respectively with *p*-chloroaniline.

Synthesis of compounds (1a-d): *N*-(2-(Pyrrolidyl)ethyl)-5-bromo indol-2,3-dione (**1a**) was synthesized by alkylation of 5-bromo isatin with (pyrrolidyl)ethyl chloride. The solution of 5-bromo-isatin (4.42 mmol, 1 g) in acetonitrile (~70 mL) was in added alumina/KF (40.7 mmol, 6.48 g) and the mixture was stirred for 5 min until brownish colour obtained. Then pyrrolidyl ethyl chloride (1.5 equiv., 6.6 mmol, 1.49 g) was added to the bottom flask and refluxed under acetonitrile for 8-10 h. The mixture was then cooled to room temperature and the suspended alumina/KF was filtered from the solution. Then, the filtrate was evaporated under reduced pressure to afford a solid which was recrystallized from hexane/chloroform (1:1) to afford the product (**Scheme-I**).

Synthesis of *N*-(4-hydroxyphenylmethyl)-5-bromo indol-2,3-dione (2): Compound **2** was synthesized by alkylation of 5-bromo isatin with 4-hydroxybenzyl chloride. The solution of 5-bromo isatin (4.42 mmol, 1 g) in acetonitrile (~70 mL) was added in alumina/KF (40.7 mmol, 6.48 g) and the mixture was stirred for 5 min until brownish colour obtained. Then 4-hydroxy benzyl chloride (1.5 equiv., 6.6 mmol, 1.49 g) was added to the bottom flask and then refluxed in acetonitrile medium for 8-10 h. The mixture was then cooled to room temperature and the suspended alumina/KF was filtered from the solution. The filtrate was allowed to evaporate under



Scheme-I: Synthesis of compounds (**1a-d**) and compounds (**2a-d**)

reduced pressure to afford a solid which was recrystallized with hexane/chloroform (1:1) to afford the product.

Synthesis of *N*-(4-(2-(pyrrolidyl)ethoxy)phenylmethyl)-5-bromo indol-2,3-dione (2a-d): For synthesis of compound *N*-(4-(2-(pyrrolidyl)ethoxy)phenylmethyl)-5-bromo indol-2,3-dione (**2a**), a mixture of of *N*-(4-hydroxyphenylmethyl)- 5-bromo indol-2,3-dione (3 mmol, 1 g) (**2**) and 2-(pyrrolidyl)-ethyl chloride (9 mmol, 1.2 mL) and KOH (6 mmol, 240 mg) was stirred for 16 h (**Scheme-I**).

Synthesis of compounds (3a-d): For synthesis of 3-(4-chloro phenylimino)-*N*-(2-(pyrrolidyl)ethyl)-5-bromo indol-2-one (**3a**), *N*-(2-(pyrrolidyl)ethyl)-5-bromo indole- 2,3-dione (**1a**, 1 mmol) and *p*-chloro phenylamine (1.2 mmol) were dissolved in minimum amount of ethanol and refluxed for 3 h with glacial acetic acid. After approximately 24 h, the products were seprated by filtration and recrystallized from warm ethanol (**Scheme-II**).

Synthesis of 3-(4-chloro phenylimino)-*N*-(4-(pyrrolidyl-ethoxy)phenyl methyl)-5-bromo indol-2-one (4a-d): For synthesis of 3-(4-chloro phenylimino)-*N*-(4-(pyrrolidyl ethoxy)-phenyl methyl)-5-bromo indol-2-one (**4a**), *N*-(4-(2-(pyrrolidyl)-ethoxy)phenylmethyl)-5-bromo indol-2,3-dione (**2a**, 1 mmol) and *para*-chloro phenylamine (1.2 mmol) were dissolved in the minimum amount of ethanol and then refluxed for 3 h with glacial acetic acid. After approximately 24 h, the products were separated by filtration and recrystallized from warm ethanol (**Scheme-III**).

Spectral data

***N*-(2-(Pyrrolidyl)ethyl)-5-bromo indol-2,3-dione (1a):** Yield: 70%, m.p.: 139-140 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.0-3.06 (s, 2H, CH_2), 2.50-2.62 (s, 2H, CH_2), 1.45-1.59 (d, 4H, pyrrolidyl), 2.00-2.25 (d, 4H, pyrrolidyl), 7.50-7.72 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.30-7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.40-7.96 (s, 1H, CH, Ar-H, 5-bromo indol-2,3-dione); FAB-MS: *m/z* 322. Anal. calcd. (found) % for $C_{14}H_{15}N_2O_2Br$ (m.w. 323.19): C, 52.03 (51.90); H, 4.68 (4.60); N, 8.67 (8.68); O, 9.90 (9.51); Br, 24.72 (24.06).

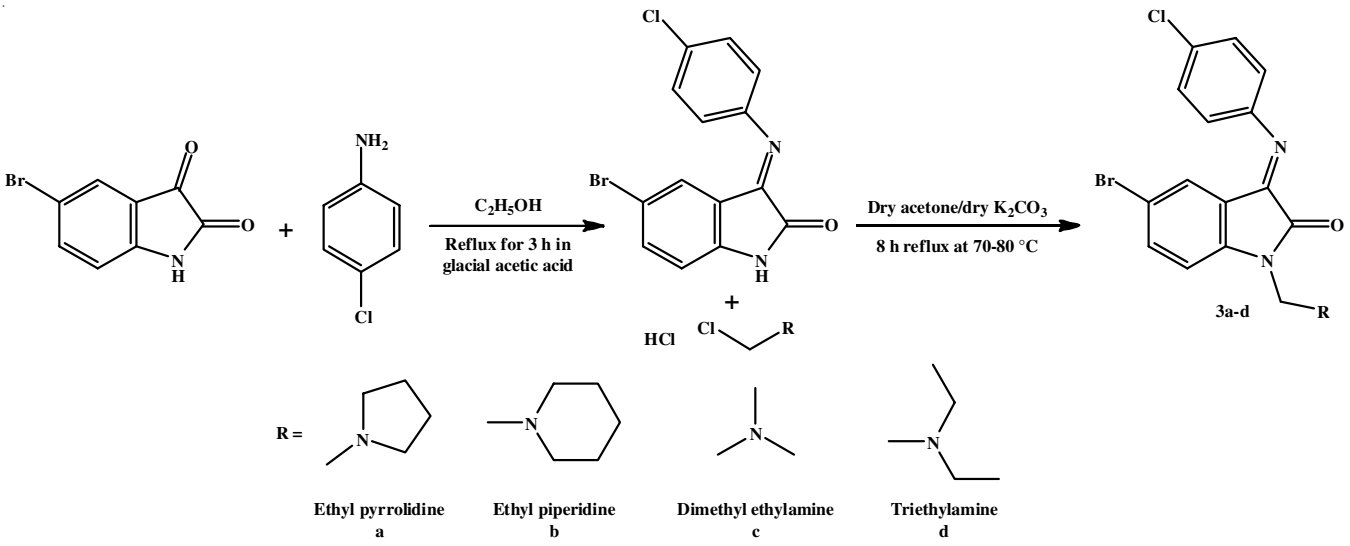
***N*-(2-(Piperidyl)ethyl)-5-bromo indol-2,3-dione (1b):** Yield: 67%, m.p.: 136-138 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.00-3.06 (s, 2H, CH_2), 2.50-2.62 (s, 2H, CH_2), 2.24 (d, 4H, piperidyl), 1.00-1.50 (t, 6H, piperidyl), 7.50-7.72 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.30-7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.40-7.96 (s, 1H, Ar-H, 5-bromo indol-2,3-dione); FAB-MS: *m/z* 322. Anal. calcd. (found) % for $C_{15}H_{17}N_2O_2Br$ (m.w. 337.21): C, 53.43 (52.90); H, 5.08 (5.43); Br, 23.70 (23.06); N, 8.31 (9.22); O, 9.49 (9.11).

***N*-(2-(Dimethylamino)ethyl)-5-bromo indol-2,3-dione (1c):** Yield: 76%, m.p.: 125-127 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.00-3.06 (s, 2H, CH_2), 2.50-2.62 (s, 2H, CH_2), 1.45-2.27 (s, 3H, CH_3 dimethylamino), 2.00-2.27 (s, 3H, CH_3 dimethyl-amino), 7.50-7.72 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.30 -7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.40-7.96 (s, 1H, Ar-H, 5-bromo indol-2,3-dione); FAB-MS: *m/z* 322. Anal. calcd. (found) % for $C_{12}H_{13}N_2O_2Br$ (m.w. 297.15): C, 48.50 (50.90); H, 4.41 (4.50)N, 8.67 (8.45); O, 9.43 (9.33); ; Br, 26.89 (25.06).

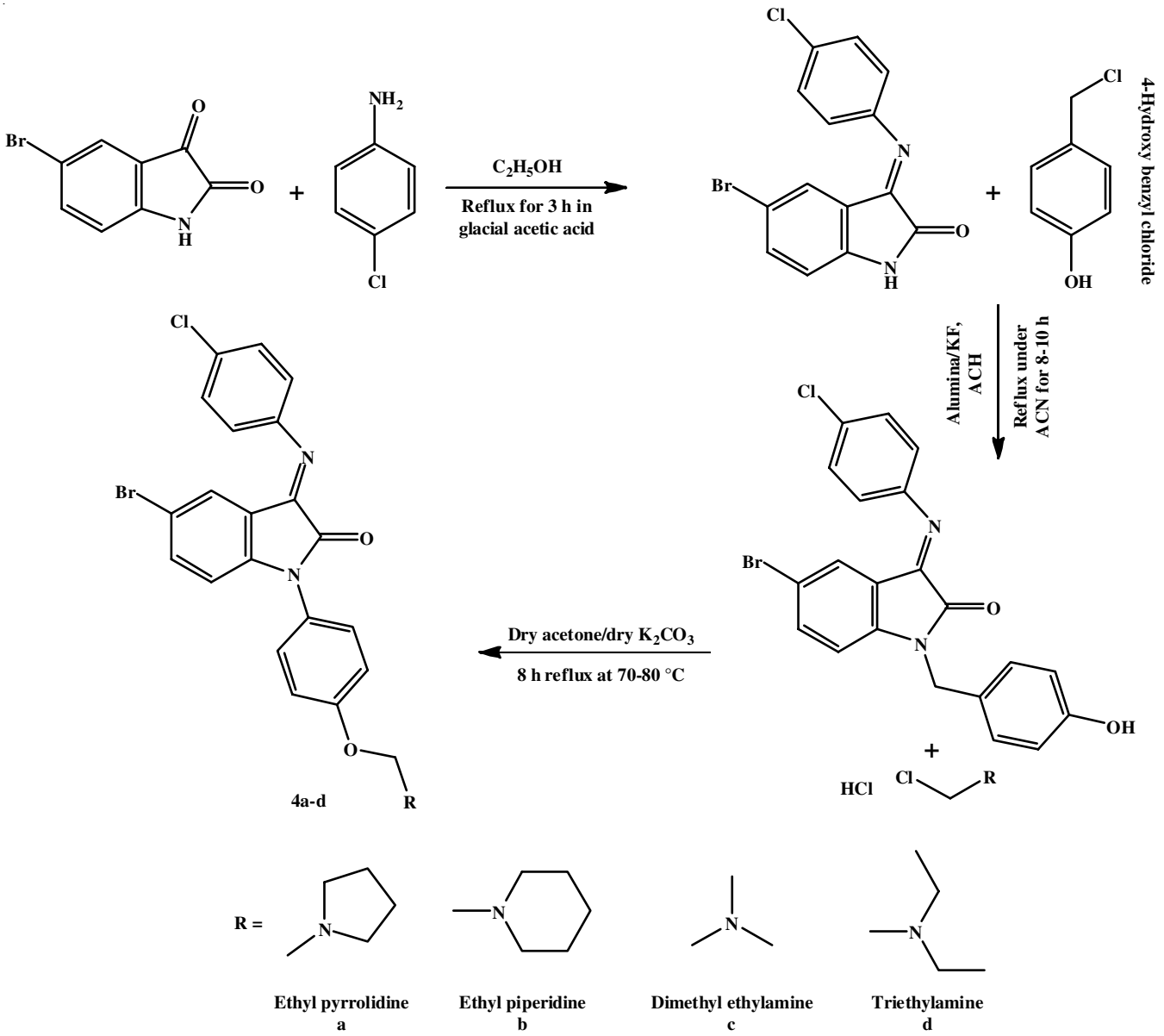
***N*-(2-(Diethyl amino)ethyl)-5-bromo indol-2,3-dione (1d):** Yield: 73%, m.p.: 128-130 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.00-3.06 (s, 2H, CH_2), 2.50-2.62 (s, 2H, CH_2), 1.45-2.40 (d, 4H, diethylamino), 1.00 (d, 6H, diethyl amino), 7.50 -7.72 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.30-7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.40-7.96 (s, 1H, Ar-H, 5-bromo indol-2,3-dione); FAB-MS: *m/z* 322. Anal. calcd. (found) % for $C_{14}H_{17}N_2O_2Br$ (m.w. 325.20): C, 51.71 (51.80); H, 5.27 (5.11); N, 8.61 (8.64); O, 9.84 (9.44); Br, 24.57 (24.06).

***N*-(4-Hydroxy phenylmethyl)-5-bromo indol-2,3-dione (2):** Yield: 80%, m.p.: 152-156 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 6.60-6.61 (d, 2H, Ar-H, phenyl), 6.60-6.89 (d, 2H, Ar-H, phenyl), 4.00-4.22 (s, 2H, CH_2), 7.50-7.72 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.30-7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.40-7.96 (s, 1H, Ar-H, 5-bromo indol-2,3-dione); FAB-MS: *m/z* 330. Anal. calcd. (found) % for $C_{15}H_{10}NO_3Br$ (m.w. 332.15): C, 54.24 (54.95); H, 3.03 (3.30); N, 4.22 (4.78); O, 14.45 (14.50); Br, 24.06 (24.67).

***N*-(4-(2-(Pyrrolidyl)ethoxy)phenylmethyl)-5-bromo indol-2,3-dione (2a):** Yield: 76%, m.p.: 102-104 °C; ^{1}H NMR



Scheme-II: Synthesis of compounds (**3a-d**)



Scheme-III: Synthesis of compounds (**4a-d**)

(200 MHz, $CDCl_3$) δ ppm: 3.0-4.04 (s, 2H, CH_2), 2.50-2.78 (s, 2H, CH_2), 1.45-1.59 (d, 4H, pyrrolidyl), 2.00-2.25 (d, 4H, pyrrolidyl), 6.60-6.65 (d, 2H, Ar-H, phenyl), 6.90-6.95 (d, 2H, Ar-H, phenyl), 4.00-4.22 (s, 2H, CH_2), 7.50-7.72 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione), 7.30-7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.40-7.96 (s, 1H, Ar-H, 5-bromo indole- 2,3-dione); FAB-MS: *m/z* 428. Anal. calcd. (found) % for $C_{21}H_{21}N_2O_3Br$ (m.w. 429.31): C, 58.75 (57.95); H, 4.93 (4.40); N, 6.53 (6.44); O, 11.18 (10.95); Br, 18.61 (18.14).

***N*-(4-(2-(Piperidyl)ethoxy)phenylmethyl)-5-bromo indol-2,3-dione (2b):** Yield: 78%, m.p.: 99-101 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.00-4.04 (s, 2H, CH_2),2.50-2.78 (s, 2H, CH_2), 2.20-2.24 (d, 4H, piperidyl), 1.30-1.50 (t, 6H, piperidyl), 6.60-6.65 (d, 2H, Ar-H, phenyl), 6.85-6.95 (d, 2H, Ar-H, phenyl), 4.00-4.22 (s, 2H, CH_2), 7.50-7.72 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.30-7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.40-7.96 (s, 1H, Ar-H, 5-bromo indol-2,3-dione); FAB-MS: *m/z* 442. Anal. calcd. (found) % for $C_{22}H_2N_2O_{33}Br$ (m.w. 443.33): C, 59.60 (53.44); H, 5.23 (5.11) N, 6.32 (6.21); O, 10.83 (10.67); Br, 18.02 (18.01).

***N*-(4-(2-(Dimethylamino)ethoxy)phenylmethyl)-5-bromo indol-2,3-dione (2c):** Yield 76%, m.p.: 89-90 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.00-4.04 (s, 2H, CH_2), 2.50-2.78 (s, 2H, CH_2), 2.50-2.27 (d, 6H, dimethylamino), 6.65 (d, 2H, Ar-H, phenyl), 6.95 (d, 2H, Ar-H, phenyl), 4.00-4.22 (s, 2H, CH_2), 7.50-7.72 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.30-7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione),7.40-7.96 (s, 1H, Ar-H, 5-bromo indol-2,3-dione); FAB-MS: *m/z* 402. Anal. calcd. (found) % for $C_{19}H_{19}N_2O_3Br$ (m.w. 403.27): C, 56.59 (56 44); H, 4.75 (4.22); N, 6.95 (6.21); O, 11.90 (11.67); Br, 19.81 (19.22).

***N*-(4-(2-(Diethylamino)ethoxy)phenylmethyl)-5-bromo indol-2,3-dione (2d):** Yield: 79%, m.p.: 92-94 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 3.00-4.04 (s, 2H, CH_2), 2.50-2.78

(s, 2H, CH_2), 2.50-2.40 (d, 4H, diethylamino), 1.00-1.00 (t, 6H, diethylamino), 6.65 (d, 2H, Ar-H, phenyl), 6.95 (d, 2H, Ar-H, phenyl), 4.00-4.22 (s, 2H, CH_2), 4.00-4.22 (s, 2H, CH_2),7.50-7.72 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.30-7.69 (s, 1H, Ar-H, 5-bromo indol-2,3-dione), 7.40-7.96 (s, 1H, Ar-H, 5-bromo indol-2,3-dione); FAB-MS: *m/z* 430. Anal. calcd. (found) % for $C_{21}H_{23}N_2O_3Br$ (m.w. 431.32): C, 58.48 (58.44); H, 5.37 (5.11); N, 6.49 (6.21); O, 11.13 (11.67); Br, 18.53 (18.33).

3-(4-Chloro phenylimino)-*N*-(2-(pyrrolidyl)ethyl)-5-bromo indol-2-one (3a): Yield: 64%. b.p.: 142-144 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 7.0-7.2 (m, 2H, chorobenzene), 7.1-7.3 (m, 2H, chlorobenzene), 3.0-3.06 (s, 2H, CH_2), 2.50-2.62 (s, 2H, CH_2), 1.50-1.59 (d, 4H, pyrrolidyl), 2.00-2.25 (d, 4H, pyrrolidyl), 7.50-7.77 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.30-7.44 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.40-7.56 (s, 1H, Ar-H, 5-bromo indol-2-one); FAB-MS: *m/z* 433. Anal. calcd. (found) % for $C_{20}H_{19}N_3OBrCl$ (m.w. 432.74): C, 55.51 (55.50); H, 4.41 (4.39); N, 9.71 (9.75); O, 3.70 (3.75); Br, 18.46 (18.42); Cl, 8.19 (8.22).

3-(4-Chloro phenylimino)-*N*-(2-(piperidyl)ethyl)-5-bromo indol-2-one (3b): Yield: 66% , b.p.: 137-139 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 7.0-7.2 (m, 2H, chloro benzene), 7.1-7.3 (m, 2H, chloro benzene), 3.0-3.06 (s, 2H, CH_2), 2.50-2.62 (s, 2H, CH_2), 1.45-1.50 (t, 6H, piperidyl), 2.00-2.24 (d, 4H, piperidyl), 7.50-7.77 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.30-7.44 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.40-7.56 (s, 1H, Ar-H, 5-bromo indol-2-one); FAB-MS: *m/z* 433. Anal calcd. (found) % for $C_{21}H_{21}N_3OBrCl$ (m.w. 446.77): C, 56.46 (55.90); H, 4.74 (4.70); N, 9.41 (9.68); O, 3.58 (3.59); Br, 17.88 (17.56); Cl, 7.94 (7.89).

3-(4-Chloro phenylimino)-*N*-(2-(dimethylamino)ethyl) 5-bromo indol-2-one (3c): Yield: 67%, b.p.: 128-130 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 7.0-7.2 (m, 2H, chloro benzene), 7.1-7.3 (m, 2H, chloro benzene), 7.1-7.3 (m, 2H, chloro benzene), 3.0-3.06 (s, 2H, CH_2), 2.50-2.62 (s, 2H, CH_2), 2.00-2.27(d, 4H dimethyl-amino),7.50-7.77 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.30 -7.44 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.40 -7.56 (s, 1H, Ar-H, 5-bromo indol-2-one); FAB-MS: *m/z* 433. Anal. calcd. (found) % for $C_{18}H_{17}N_3OBrCl$ (m.w. 406.70): C, 53.16 (53.51); H, 4.21 (4.41); N, 10.33 (10.11); O, 3.93 (3.70); Br, 19.65 (19.22); Cl, 8.72 (8.19).

3-(4-Chloro phenylimino)-*N*-(2-(diethylamino)ethyl)-5-bromo indol-2-one (3d): Yield: 71%. b.p.: 133-135 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 7.0-7.2 (m, 2H, chloro benzene), 7.1-7.3 (m, 2H, chloro benzene), 3.0-3.06 (s, 2H, CH_2), 2.50-2.62 (s, 2H, CH_2), 2.00-2.40 (d, 4H dimethylamino), 1.00 (d, 6H, diethylamino), 7.50-7.77 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.30-7.44 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.40-7.56 (s, 1H, Ar-H, 5-bromo indol-2-one); FAB-MS: *m/z* 433. Anal. calcd. (found) % for $C_{20}H_{21}N_3OBrCl$ (m.w.: 434.76): C, 55.25 (55.51); H, 4.87 (4.41); Br, 18.38 (18.22); Cl, 8.15 (8.19); N, 9.67 (9.11); O, 3.68 (3.70).

3-(4-Chloro phenylimino)-*N*-(4-(pyrrolidylethoxy)-phenyl methyl)-5-bromo indol-2-one (4a): Yield 73%; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 7.0-7.2 (m, 2H, chloro benzene), 7.1 -7.3 (m, 2H, chloro benzene), 4.50-4.04 (s, 2H, CH_2), 2.00-2.78 (s, 2H, CH_2), 1.00 -1.59 (d, 4H, pyrrolidyl), 2.00-2.25 (d, 4H, pyrrolidyl), 4.00-4.22 (s, 2H, CH_2), 6.65 (d, 2H, Ar-H, phenyl), 6.95 (d, 2H, Ar-H, phenyl), 7.50 -7.77 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.30 -7.44 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.40-7.56 (s, 1H, Ar-H, 5-bromo indol-2-one); FAB-MS: *m/z* 539. Anal. calcd. (found) % for $C_{27}H_{25}N_3O_2BrCl$ (m.w. 538.86): C, 60.18 (60.45); H, 4.68 (4.70); N, 7.80 (7.48); O, 5.94 (5.33); Br,14.83 (14.22); Cl, 6.58 (6.22).

3-(4-Chloro phenylimino)-*N*-(4-(piperidyl ethoxy)-phenyl methyl)-5-bromo indol-2-one (4b): Yield: 67%; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 7.0 -7.2 (m, 2H, chloro benzene), 7.1-7.3 (m, 2H, chloro benzene), 4.50-4.04 (s, 2H, CH_2), 2.00-2.78 (s, 2H, CH_2), 2.00-2.24 (d, 4H, piperidyl), 1.00-1.50 (t, 6H, piperidyl), 4.00-4.22 (s, 2H, CH_2), 6.60 -6.65 (d,2H, Ar-H, phenyl), 6.70-6.95 (d, 2H, Ar-H, phenyl), 7.50-7.77 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.30-7.44 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.40-7.56 (s, 1H, Ar-H, 5-bromo indol-2-one); FAB-MS: *m/z* 533. Anal. calcd. (found) % for $C_{28}H_{27}N_3O_2BrCl$ (m.w. 552.89): C, 60.83 (60.45); H, 4.92 (4.70); N, 7.60 (7.48); O, 5.79 (5.33); Br, 14.45 (14.22); Cl, 6.41 (7 22).

3-(4-Chloro phenylimino)-*N*-(4-(dimethylamino ethoxy)-phenyl methyl)-5-bromo indol-2-one (4c): Yield: 69%; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 7.0-7.2 (m, 2H, chloro benzene), 7.1-7.3 (m, 2H, chloro benzene), 4.50-4.04 (s, 2H, CH_2), 2.00 -2.78 (s, 2H, CH_2), 2.20-2.27 (d, 6H, CH_3, dimethylamino), 4.00-4.22 (s, 2H, CH_2), 6.65 (d, 2H, Ar-H, phenyl), 6.95 (d, 2H, Ar-H, phenyl), 7.50-7.77 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.30-7.44 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.40-7.56 (s, 1H, Ar-H, 5-bromo indol-2-one); FAB-MS: *m/z* 513. Anal. calcd. (found) % for $C_{25}H_{23}N_3O_2BrCl$ (m.w. 512.83): C, 58.55 (58.10); H, 4.52 (4.40); N, 8.19 (8.01); O, 6.24 (6.23); Br, 15.58 (15.22); Cl, 6.91 (6.90).

3-(4-Chloro phenylimino)-*N*-(4-(dimethylamino-ethoxy)-phenyl methyl)-5-bromo indol-2-one (4d): Yield: 68%; ^{1}H NMR (200 MHz, $CDCl_3$) δ ppm: 7.0-7.2 (m, 2H, chloro benzene), 7.1-7.3 (m, 2H, chloro benzene), 4.50-4.04 (s, 2H, CH_2), 2.00 -2.78 (s, 2H, CH_2), 2.00-2.40 (d, 4H, CH_2, diethylamino), 1.00 (d, 6H, CH_3, diethylamino), 4.00-4.22 (s, 2H, CH_2), 6.65 (d, 2H, Ar-H, phenyl), 6.95 (d, 2H, Ar-H, phenyl), 7.50-7.77 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.30 -7.44 (s, 1H, Ar-H, 5-bromo indol-2-one), 7.40-7.56 (s, 1H, Ar-H, 5-bromo indol-2-one); FAB-MS: *m/z* 541. Anal. calcd. (found) % for $C_{27}H_{27}N_3O_2BrCl$ (m.w. 540.88): C,59.96 (59.88); H, 5.03 (5.01); N, 7.77 (7.65); O, 5.92 (5.66); Br, 14.77 (14.23); Cl, 6.55 (6.56).

Biological activities

***in silico* Molecular docking studies:** Molecular docking of compounds **1a**, **2a**, **3a**, **4a** and standard anticancer drug doxorubicin were performed through the AutoDock Vina, an open-source software, which shows 78% binding mode prediction accuracy on test set (Molecular Graphics Lab at The Scripps Research Institute, USA) [27]. During docking process, all the parameters were kept on default values by selecting the full grid size of protein. The docking poses were visualized by using Discovery Studio Visualizer software. Before docking, all the designed compounds were drawn and energy minimized using ChemOffice 2002. Three-dimensional (3D) crystallographic structure of human aldehyde dehydrogenase: ALDH2

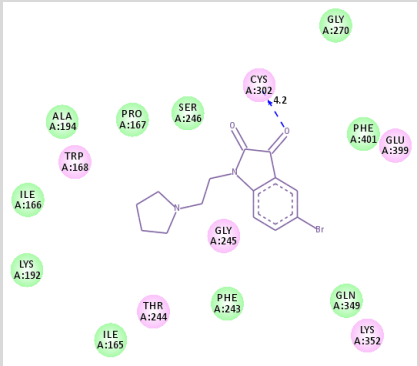
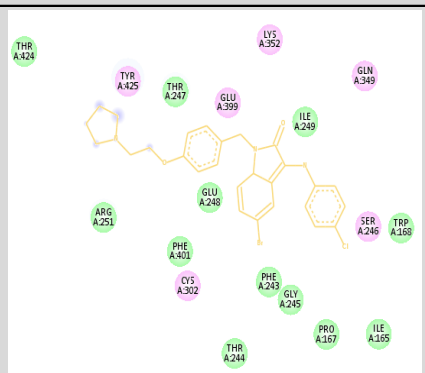
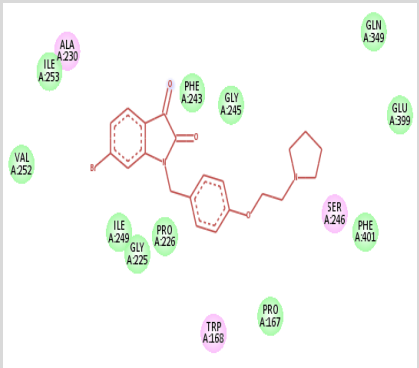
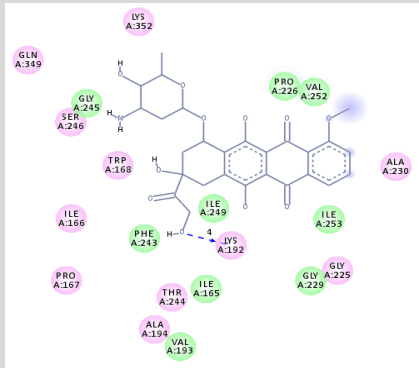
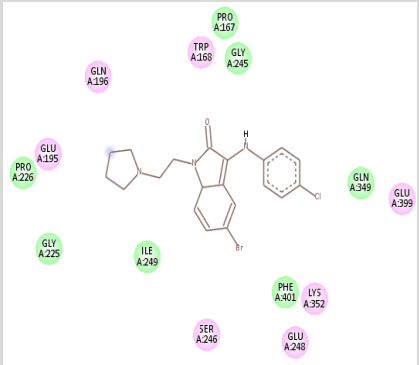
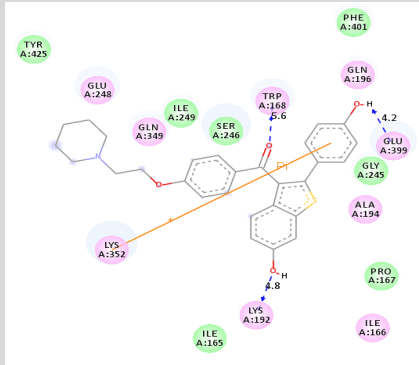
(PBD ID: 5L13) [28] was downloaded from RCSB PDB (https://www.rcsb.org/structure/5L13), which is also reported as one of the breast cancer (MCF-7 cell line) protein target. All the ligands and protein 3D structure were prepared before docking as per standard protocol by removing water molecules and adding hydrogen to protein and ligands.

The protein structure for docking study was predicted/identified using STITCH online server (http://stitch.embl.de/), by submitting the SMILE of designed ligands. The STITCH server works on the method of searching the query compound with the database compound and find the similar structure on the basis of structure similarity and gave tanimoto score more than 70%. We selected the matched compound (STITCH ID: R3739, which is active against ALDH2.

***in vitro* Antibacterial activity:** The antibacterial activity was determined by agar disc diffusion method on three different bacterial strains *viz. P. aeruginosa*, *S. aureus* and *K. pneumonia* with concentration 10 µg/mL. Test solution was prepared in dimethylsulfoxide (DMSO) with 3.8 g of Mueller Hinton agar dissolved in distilled water (100 mL) and sterilized by autoclaving at 120 ºC for 15 min. The discs were put on plates and incubated at 37 ºC for 18-24 h. Finally, the zones of inhibition were measured in mm.

***in vitro* Antifungal activity:** For antifungal assay, agar disc diffusion method was used and tested on *Aspergillus flavus* and *Aspergillus niger* with concentration 10 µg/mL. Stock solutions of the test compounds were prepared in dimethylsulfoxide (DMSO). The Sabouraud dextrose agar was used for petri-plate preparation and 3.8 g of Sabouraud dextrose agar was dissolved in distilled water (100 mL) and sterilized at 120 ºC for 15 min. The applied discs plates were incubated at 280 ºC for 72-96 h and then the zones of inhibition were measured in mm.

TABLE-1
MOLECULAR DOCKING RESULTS, COMPOUNDS NAME, DOCKING 3 DIMENSIONAL (3D) POSES AND DOCKING ENERGY

Compd.	Docking 3D poses	Docking energy (Kcal/mol)	Compd.	Docking 3D poses	Docking energy (Kcal/mol)
1a		-7.7	**4a**		-8.8
2a		-8.2	Doxorubicin		-8.9
3a		-7.7	Raloxifene hydrochloride		-7.8

RESULTS AND DISCUSSION

The potential isatin derivatives based on the isatin scaffold in combination with other pharmacophoric moieties of proven drugs were synthesized, characterized and evaluated for various biological activities. For this, 5-bromo isatins was used as starting material considering its superior biological activities compare to substituted isatin with other groups such as -Cl, -OH, *etc*. Compounds of series **3a-d** and **4a-d** were synthesized by imine formation at C_3 with alkylation at *N*-position to enhance antibacterial and antifungal activities.

***in-silico* Antitumor activity:** The docking study was performed to identify the binding conformation of derived compounds against the MCF-7 cancer cell-line. The docking study revealed that all the derived compounds showed good binding affinity as compared to the standard drugs. From Table-1, it is clear that the compound **4a** shows a binding affinity nearly to doxorubicin (a potent anticancer drug), while other compounds and the standard drugs sharing same protein binding pocket. Fig. 1 shows the 3D representation the docking pose of the synthesized compounds with two control drugs.

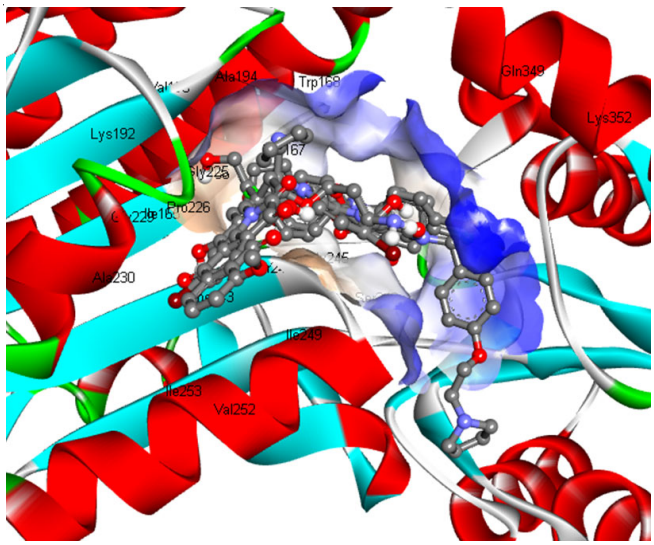


Fig. 1. 3D Docking pose of four derivatives (**1a**, **2a**, **3a**, **4a**) with two control drugs (doxorubicin, raloxifene hydrochloride). All the ligands bind in the same binding pocket of protein (aldehyde dehydrogenase 2: ALDH2) PDB ID: 5L13

***in vitro* Antitumor activity:** *in vitro* Antitumor activities of the synthesized compounds **3a-d** and **4a-d** were evaluated against MCF-7 and EVSA-T cell lines. Compound **3c** was found to possess superior antitumor activity against both MCF-7 and EVSA-T cell lines while compounds **4a** and **4d** possess better anti-tumor activity against both MCF-7 and EVSA-T cell lines (Table-2).

Antibacterial activity: The synthesized compounds **3a-d** and **4a-d** were evaluated for their antibacterial activity against *P. aeruginosa*, *S. aureus* and *K. pneumonia* strains at 10 µg/mL test solution. Compounds of series (**3a-d**) exhibited excellent antibacterial activity against *S. aureus*, *P. aeruginosa* and *K. Pneumoniae* with zone of inhibition in the range of 10-14 mm except compound **3d** against *K. pneumoniae* (Table-3). Similarly,

TABLE-2
ANTITUMOR ACTIVITY OF COMPOUNDS (**3a-d** AND **4a-d**) ON CELL LINES MCF-7 AND EVSA-T

Compounds	MCF-7 (Cell No. × 10^4)	EVSA-T (Cell No. × 10^4)	Activity
3a	8.95 ± 0.67	8.55 ± 0.62	Positive
3b	8.79 ± 0.52	8.42 ± 0.46	Positive
3c	9.19 ± 0.92	9.29 ± 0.88	Positive
3d	8.95 ± 0.67	8.55 ± 0.62	Positive
4a	9.29 ± 0.88	9.89 ± 0.92	Positive
4b	8.95 ± 0.67	8.55 ± 0.62	Positive
4c	8.79 ± 0.52	8.42 ± 0.46	Positive
4d	9.29 ± 0.88	9.89 ± 0.92	Positive

compounds (**4a-d**) series, also exhibited the excellent antibacterial activity against *P. aeruginosa*, *K. pneumoniae* and *S. aureus* with zone Inhibition (ZI) in the range of 10-14 mm (Table-3). Compounds **4a** and **4c** were found to possess superior antibacterial activity with ZI > 14 mm against *K. pneumoniae* and *P. aeruginosa*, respectively (Table-3).

TABLE-3
ANTIBACTERIAL ACTIVITY FOR COMPOUNDS (**3a-d** and **4a-4d**)

	Antibacterial activity @ 10 µg/mL concentration		
Compounds	*P. aeruginosa*	*S. aureus*	*K. pneumoniae*
3a	++	++	++
3b	+++	+++	++
3c	++	++	+++
3d	++	++	+
4a	++	++	+++
4b	++	++	++
4c	+++	++	+
4d	++	++	++

+ Slightly active (diameter range 6-10 mm)
++ Moderately active (diameter range 10-14 mm)
+++ Highly active (diameter range > 14 mm)

Antifungal activity: Compounds **3a-d** and **4a-d** were also screened for their antifungal activity against *Aspergillus flavus* and *Aspergillus niger* in terms of zone of inhibition (%). It was found that some compounds of both series **3a-d** and **4a-d** exhibit promising antifungal properties. Compounds **3a** and **3d** display the superior activity against both *A. flavus* and *A. niger*, while compounds **3b** and **3c** performed better antifungal activity against *Aspergillus niger* (Table-4). Among the compounds of series **4a-d**, compounds **4a** and **4c** show better antifungal activity against both *Aspergillus flavus* and *Aspergillus niger*. However, compound **4b** showed good antifungal activity against *A. niger* (Table-4).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

TABLE-4
ANTIFUNGAL ACTIVITY ON *A. flavus* AND *A. niger*
FOR COMPOUNDS (**3a-d** AND **4a-d**)

Antifungal activity @ 10 µg/mL concentration		
Compounds	*A. flavus*	*A. niger*
3a	0.7 ± 0.004	0.6 ± 0.004
3b	0.2 ± 0.003	0.7 ± 0.004
3c	0.2 ± 0.003	0.7 ± 0.004
3d	0.7 ± 0.004	0.7 ± 0.004
4a	0.8 ± 0.004	0.8 ± 0.004
4b	0.3 ± 0.003	0.7 ± 0.004
4c	0.8 ± 0.004	0.8 ± 0.004
4d	0.2 ± 0.003	0.5 ± 0.003
Col. dia. (mm)		

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