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DIVERSITY ORIENTED DESIGN OF VARIOUS HYDRAZIDES AND AMIDES DERIVED FROM ISATOIC ANHYDRIDE AND THEIR ANTIMICROBIAL EVALUATION

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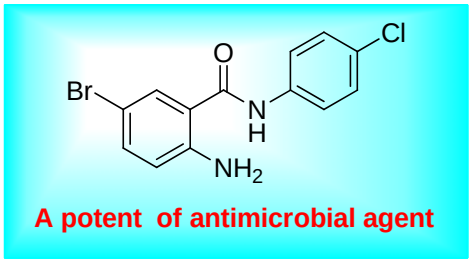
In this work, synthesis and biological evaluation of series of hydrazides **2a-e** and amides **4a-d**, **6a-g** functionality have been reported. All sixteen compounds were synthesized using short and convenient one high yielding step starting from isatoic anhydride derivatives. Some newly synthesized compounds were subjected to *in-vitro* antibacterial and antifungal screening. Compound **4b** could be considered as the most active members in this investigation with a broad spectrum of antibacterial activity against two types of Gram-positive bacteria, two types of Gram-negative bacteria together with an appreciable antifungal activity against A. fumigatus, S. cerevisiae and C. albicans. This compound found to be more potent than streptomycin against B. subcilils and Escherichia coli. Neversless, **4b** showed greater effect than well known antifungal agent such as clotrimazole against A. fumigatus and can serve as potential lead molecules for further investigation.



Graphical abstract

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1. Introduction

The increasing drug resistance of Gram-positive and Gram-negative pathogenic bacteria to the currently marketed antibacterial agents has caused alarm in the international scientific community [1]. Subsequently, there is an urgent need for the development of new drug molecules

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with newer targets, more potent, selective non-traditional antimicrobial agents and with an alternative mechanism of action. One of the challenge is that hydrazides which have received considerable attention in last decade due to their usefulness in different area of chemothearpy and as industerial builidng blocks. Hydrazides and hydrazones have been demonstrated to possess antiinflammatory, antitubercular, anticonvulsant, antiplatelet, analgesic, antitumoral activities [2,3], and antimicrobial [4]. Recent advances in biological research, isatoic anhydride has been used as an intermediate for pharmaceutically important molecules [5,6], among various of heterocycles have been synthesised from it, for example quinazolones, quinazolinones, phthalimides, benzimidazolones, quinazolinediones, pyrroloquinazolones, in the fluorescent labeling of tRNA and mRNA [7-11],

Encouraged by these observations and in continuation of our previous work [12-21] we are interested in identification of new valuable candidates in designing new, less toxic and potent antimicrobial agents.

In this text, we here documented the synthesis, characterization, and antimicrobial activity of series of hydrazides and amides starting from isatoic anhydrides. The methodology depends on the ring opening of isatoic anhydride with *N*-nucleophile.

2. Experimental Section

M.P. was measured on a Gallenkamp melting point apparatus in open glass capillaries and are uncorrected. IR spectra were measured as KBr pellets on a Perking Elmer FT 1000 spectrophotometer. The NMR spectra were recorded on a Varian Mercury Jeol-400 NMR spectrometer.^{1}H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) were run in (DMSO-d_6). Chemical shifts (δ) are referred in ppm and coupling constants *J* are given in Hz. Abbreviations for multiplicity are as follows: s (singulet), d (doublet), t (triplet), q (quadruplet), m (multiplet). Mass spectra were recorded on a Shimadzu GCMS-QP 1000 EX mass spectrometer at 70 eV. Elemental analysis was carried out on an Elementar Vario EL analyzer.

General method for synthesis of 2-amino-N -(substituted benzoyl)substituted hydrazide 2a-e(GP1)

Procedure A (2a-e): To a solution of isatoic anhydride (3-4mmol) in 5-10 ml DMF were added a solution of hydrazide derivatives or hydrazine hydrate (5-10mmol) in 5-10 ml DMF and the reaction mixture was refluxed for 6 h. the reaction mixture was monitored by TLC (EtOH:$CHCl_3$ 1:9)The reaction mixture was left to cool to RT. The formed solid product was filtered off and recrystallized to afford the corresponding product **2a-e**.

Procedure B(2a,e): A mixture of isatoic anhydride (1mmol) and hydrazide derivatives (5-10mmol) in the presence of view drops from DMF, was exposed to microwave irradiation (400w) for 10 min. The reaction mixture was left to cool to RT. 5ml of cooled water was added and the formed solid product was filtered off and recrystallized afforded the corresponding product **2a,e**.

N'-(2-Aminobenzoyl)isonicotinohydrazide 2a

2a was prepared according to GP1 by method A or method B, afford **2a** as pale yellow powder; yield (67[a], 80[b] %); m.p. 198 °C; IR ν_{max} (KBr) 3431.50, 3291.65, 3186.38, 1697.60, 1663.22, 1594.23 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 10.70 (2H, br.s, 2NH), 8.79 (2H, d, *J* =5.8Hz, Ph), 7.83 (2H, d, *J* =5.8Hz, Ph), 7.62 (1H, d, *J* = 7.6Hz, Ph), 7.21 (1H, t, *J* = 7.6Hz, Ph), 6.75 (1H, d, *J* = 7.6Hz, Ph), 6.56 (1H, t, *J* = 7.6Hz, Ph), 6.45 (2H, br.s, NH_2) ;^{13}C-NMR: δ 115.2, 119.0, 121.6, 121.9, 131.6, 134.7, 139.9, 142.7, 150.9, 165.9, 170.4; MS *m/z* (%):256 [M^+] ($C_{13}H_{12}N_4O_2$) (26.56), 211 (9.37), 155 (10.94), 140 (7.81), 126 (34.37), 98 (100) ; Anal. for $C_{13}H_{12}N_4O_2$ (256.26) calcd; : C, 60.93; H, 4.72; N, 21.86; Found: : C, 60.96; H, 4.71; N, 21.87.

2-Amino-N'-(4-aminobenzoyl)benzohydrazide 2b

2b was prepared according to GP1, method A and recrystallized from methanol afford **2b** as peige powder; yield (65 %); m.p. 200°C; IR ν_{max} (KBr) 3458.71, 3412.74, 3363.91, 3281.98, 1626.79, 1606.12 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 9.88,9.97 (2H, br.s, 2NH), 7.59-7.66 (3H, m, Ph) , 7.18 (1H, t, *J* =7.8Hz, Ph), 6.72 (1H, d, *J* =7.8Hz, Ph), 6.51-6.58 (3H, m, Ph) , 5.73,6.40 (2H, br.s, NH_2)) ;^{13}C-NMR: δ 113.1, 113.5, 115.1, 116.8, 119.6, 128.7, 129.6, 132.6, 150.3, 152.7, 166.5, 169.0; MS *m/z* (%):270 [M^+] ($C_{14}H_{14}N_4O_2$) (100), 240 (10.23), 224 (23.55),

212 (6.14), 182 (33.45), 75 (13.00); Anal. for $C_{14}H_{14}N_4O_2$ (270.29) calcd; C, 62.21; H, 5.22; N, 20.73; Found: C, 62.24; H, 5.21; N, 20.74.

2-Amino-N'-(4-chlorobenzoyl)benzohydrazide 2c

2c was prepared according toGP1, method A and recrystallized from methanol afford **2c** as peige cubes; yield (74%); m.p. 210 °C; IR ν_{max} (KBr) 3412.8, 3255.90, 1677.2, 1650.77cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 10.21,10.49 (1H, br.s, 2NH), 7.94(1H, d, J =8.8Hz, Ph), 7.60-7.62(3H, m, Ph), 7.20 (1H, t, J=7.9Hz, Ph), 6.74 (1H, d, J =7.9Hz, Ph), 6.55 (1H, t, J =7.9Hz, Ph), 6.45 (2H, br.s, NH_2) ; ^{13}C-NMR: δ 112.8, 115.1, 116.9, 128.7, 129.2, 129.9, 131.9, 132.9, 137.2, 150.5, 165.5, 168.8; MS *m/z* (%): 289 [M$^+$] ($C_{14}H_{12}N_3O_2Cl$) (10.94), 257 (39.06) , 245 (100), 217 (10.94), 210 (61.54) , 202 (34.37); Anal. for $C_{14}H_{12}ClN_3O_2$ (289.72) calcd; C, 58.04; H, 4.17; Cl, 12.24; N, 14.50; Found: C, 58.10; H, 4.20; Cl, 12.23; N, 14.48;

2-Amino-N'-benzoylbenzohydrazide 2d

2d was prepared according to GP1, method A, and recrystallized from ethylacetate afford **2d** as, gray powder; yield (63%); m.p. 212 °C; IR ν_{max} (KBr) 3383.13, 3212.04 , 1736.42, 1693.80 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 7.98 (2H, d, J =6.9Hz, Ph), 7.93,7.95 (1H, br.s, 2NH), 7.74 (1H, t, J =6.9Hz, Ph), .52-7.65 (4H, m, Ph), 7.27 (1H, d, J =7.3Hz, Ph), 7.20 (1H, t, J =7.3Hz, Ph), 6.44 (2H, br.s, NH_2);^{13}C-NMR: δ 115.1, 116.1, 116.9, 128.2, 129.0, 129.2, 132.1, 132.3, 132.9, 149.5, 165.7, 166.5; MS *m/z* (%): 255 [M$^+$] ($C_{14}H1_3N_3O_2$); Anal. for $C_{14}H_{13}N_3O_2$ (255.27) calcd; C, 65.87; H, 5.13; N, 16.46; Found: C, 65.86; H, 5.13; N, 16.47

2-Aminobenzohydrazide 2e

2e was prepared according to GP1, method A or method B (10min,400W), beige powder; yield (70^{a}, 72^{b} %); m.p. 200 °C; IR ν_{max} (KBr) 3445.81, 3414.14 , 3310.80 , 1651.61cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 7.95 (1H, br.s, NH), 7.61 (1H, d, J = 8.2Hz, Ph), 7.19 (1H, t, J = 8.2Hz, Ph), 6.73 (1H, d, J = 8.2Hz, Ph), 6.54 (1H, t , J = 8.2Hz, Ph), 2.89, 6.42 (each 2H, br.s, 2NH_2) ;^{13}C-NMR: δ 113.1, 115.1, 116.9, 128.7, 132.7, 150.4, 168.9; MS *m/z* (%):151[M$^+$] ($C_7H_9N_3O$) (1.56), 73 (14.06), 68 (10.94), 57 (100) , 31 (70.77), 29 (90.77); Anal. for C7H9N3O (151.17) calcd; C, 55.62; H, 6.00; N, 27.80; Found: C, 55.66; H, 6.05; N, 27.83.

General method for synthesis of 2-Amino-N-substituted-5-bromo benzamide 4a-d(GP2)

Procedure A (4a-d): To a solution of 5-bromoisatoicanhydride (2mmol) in 2-3 ml DMF were added a solution of amine derivatives (5-10mmol) in 5-10 ml DMF and the reaction mixture was refluxed for 6 h. the reaction mixture was monitored by TLC (EtOH:$CHCl_3$ 1:9)The reaction mixture was left to cool to RT, poured into water (100-300ml). The formed solid product was filtered off and recrystallized to afford the corresponding product **4a-d**.

Procedure B (4c,d): A mixture of 8-bromoisatoicanhydride (1mmol) and amine derivatives (5-10mmol) in the presence of view drops from DMF, was exposed to microwave irradiation (140-280w) for 10 min. The reaction mixture was left to cool to RT. 5ml of cooled water was added and the formed solid product was filtered off and recrystallized afforded the corresponding product **4c,d**.

2-Amino-5-bromo-N-phenylbenzamide 4a

4a was prepared according to GP2, method A, white powder; yield (82 %); m.p. 138 °C; IR ν_{max} (KBr) 3419.58, 3289.63, 1627.70 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 7.34-7.39 (7H, m, NH, Ph), 7.24 (1H, s, Ph), 6.56 (1H, d, J = 8.8Hz, Ph), 5.56 (2H, br.s, NH_2), 4.57 (2H, d, J= 5.9Hz, CH_2);^{13}C-NMR: δ 43.9, 107.7, 117.3, 118.9, 127.7, 127.9, 128.9, 129.6, 135.0, 137.9, 147.8, 167.9; MS *m/z* (%): 304[M$^+$]($C_{14}H_{13}N_2OBr$) (10.93), 305[M$^+$+1](50.77), 303[M$^+$-1] (100), 301[M$^+$-2H](52.31), 275 (14.06), 196 (26.56); Anal. for $C_{13}H_{11}BrN_2O$ (291.14) calcd; C, 53.63; H, 3.81; Br, 27.44; N, 9.62; Found: C, 53.64; H, 3.82; Br, 27.46; N, 9.64.

2-Amino-5-bromo-N-(4-chlorophenyl)benzamide 4b

4b was prepared according to GP2, method A, shining peige powder; yield (83 %); m.p. 198 °C; IR ν_{max} (KBr) 3454.00, 3358.56 , 3299.42 , 1623.79 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 8.93 (1H, br.s, NH), 7.72 (1H, s, Ph), 7.27-7.38 (5H, m, Ph), 6.68 (1H, d, J = 8.0Hz, Ph), 6.62 (2H, br.s, NH_2), 4.39 (2H, s, CH_2);^{13}C-NMR: δ 40.3, 105.4, 116.1, 119.1, 128.7, 129.7, 130.7, 131.8, 134.9, 139.3, 149.6, 168.1; MS *m/z* (%):338 [M$^+$] ($C_{14}H_{12}N_2OBrCl$) (58.46), 336 [M$^+$-2H] (31.25) , 278 (100), 276 (50.77), 265 (96.92) , 263 (50.77) ; Anal. for $C_{13}H_{10}BrClN_2O$ (325.59)

calcd; C, 47.96; H, 3.10; Br, 24.54; Cl, 10.89; N, 8.60; Found: C, 47.96; H, 3.08; Br, 24.44; Cl, 10.88; N, 8.59.

2-Amino-5-bromo-N-(3,4-dimethoxybenzyl)benzamide 4c

4c was prepared according to GP2 ,method A, or method B(6min, 280w), white scales; yield (78[a],70[b] %); m.p. 135 °C; IR ν_{max} (KBr) 3461.94, 3345.00, 3146.35, 1654.99cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 8.38 (1H, br.s, NH), 7.56 (1H, s, Ph), 7.24 (1H, s, Ph), 6.67-6.95 (4H, m, Ph), 6.52 (2H, br.s, NH_2), 3.71 (6H, s, $2OCH_3$), 2.75-3.39 (4H, m, $2CH_2$);^{13}C-NMR: δ 39.9, 40.5, 55.9, 56.0, 105.4, 112.5, 113.1, 116.9, 118.9, 121.0, 130.7, 132.6, 134.5, 147.8, 149.1, 149.3, 168.0; MS *m/z* (%): 378 [M$^+$] ($C_{17}H_{19}N_2O_3Br$) (0.2), 377 [M$^+$-1] (1.60), 336 [M$^+$-2H] (0.20) , 301 (12.50), 300 (100), 298 (51.00). Anal. For $C_{16}H_{17}BrN_2O_3$ (365.22) calcd; C, 52.62; H, 4.69; Br, 21.88; N, 7.67; Found: C, 52.64; H, 4.70; Br, 21.89; N, 7.65.

2-amino-5-bromo-N-(3,4-dimethoxyphenyl)benzamide 4d

4d was prepared according to GP2 ,method A, or method B(6min, 280w), black powder; yield (68[a],60[b] %); m.p. 181 °C; IR ν_{max} (KBr) 3439.09, 3322.6, 3372.00, 1645.64 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 9.96 (1H, br.s, NH), 7.99 (1H, s, Ph), 7.89 (1H, d,^3J = 8.8Hz, Ph), 7.37 (1H, s , Ph), 7.26 (1H, d, *J*= 8.0Hz, Ph), 6.91(1H, d, *J* = 8.0Hz, Ph), 6.72 (1H, d, *J*= 8.8Hz, Ph), 6.48 (2H, br.s, NH_2), 3.74 (6H, s, $2OCH_3$);^{13}C- NMR: δ 56.0, 56.12 ,105.5, 112.3, 113.1, 117.3, 119.0, 131.0, 131.2, 133.0, 134.9, 145.7, 148.9, 149.4, 166.7; MS *m/z* (%): 350 [M$^+$] ($C_{15}H_{15}BrN_2O_3$) (63.74), 351[M$^+$+1](100), 307 (88.90), 306 (72.04), 270 (32.33), 222 (74.69); Anal. for $C_{15}H_{15}BrN_2O_3$ (351.20) calcd; C, 51.30; H, 4.31; Br, 22.75; N, 7.98; Found: C, 51.32; H, 4.33; Br, 22.77; N, 7.99.

General method for synthesis of 2-Amino- N- substituted- 3-Chloro – benzamide 6a-g (GP3)

Procedure A (6a-g): To a solution of 10-chlorooisatoicanhydride **5** (2mmol) in 2-3 ml DMF were added a solution of amine derivatives (5-10mmol) in 5-10 ml DMF and the reaction mixture was refluxed for 6 h. the reaction mixture was monitored by TLC (EtOH:$CHCl_3$ 1:9)The reaction mixture was left to cool to RT, poured into water (100-300ml). The formed solid product was filtered off and recrystallized to afford the corresponding product **6a-g**.

Procedure B (6c-g): A mixture of 10-chloroisatoicanhydride **5** (1mmol) and amine derivatives (5-10mmol) in the presence of view drops from DMF, was exposed to microwave irradiation (140-300w) for 4-8 min. The reaction mixture was left to cool to RT. 5ml of cooled water was added and the formed solid product was filtered off and recrystallized afforded the corresponding product **6c-g**.

2-Amino-N-benzyl-3-chlorobenzamide 6a

6a was prepared according to GP3, method A, afford **6a** as beige powder; yield (98[a]%); m.p. 108°C; IR ν_{max} (KBr) 3494.74, 3390.77 , 3288.53 , 1627.70cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 9.00 (1H, br.s, NH), 7.58 (1H, d, *J* = 8.0Hz, Ph), 7.31-7.40 (6H, m, Ph), 6.59 (1H, t, *J* = 8.0Hz, Ph), 6.55 (2H, br.s, NH_2), 4.44 (2H, s, CH_2);^{13}C-NMR: δ 42.9, 115.8, 117.1, 119.6, 127.3, 127.5, 127.7, 128.8, 132.3, 140.1, 145.8, 168.7; MS *m/z* (%):260 [M$^+$] ($C_{14}H_{13}ClN_2O$); Anal. for $C_{14}H_{13}ClN_2O$ (260.72) calcd; C, 64.49; H, 5.03; Cl, 13.60; N, 10.74; Found: C, 64.50; H, 5.10; Cl, 13.57; N, 10.73.

2-Amino-N-benzyl-3-chlorobenzamide 6b

6b was prepared according to GP3, method A, afford **6b** as beige needles; yield (75[a]%); m.p. 148°C; IR ν_{max} (KBr) 3474.33, 3352.59 , 3227.20, 1631.22cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 8.51(1H, br.s, NH), 7.62 (1H, d, *J* = 7.8Hz, Ph), 7.59 (2H, d, *J* = 8.4Hz, Ph), 7.43 (1H,d , *J* =7.8 Hz, Ph), 7.15 (2H, d, *J* = 8. 4Hz, Ph), 6.66 (1H, t, *J* = 7.8Hz, Ph), 6.38 (2H, br.s, NH_2), 2.28 (3H, s, CH_3);^{13}C-NMR: δ 21.0, 115.9, 119.5, 121.3, 128.3, 129.2, 129.5, 132.5, 133.3, 136.9, 145.6, 167.5; MS *m/z* (%):260 [M$^+$] ($C_{14}H_{13}ClN_2O$); Anal. for $C_{14}H_{13}ClN_2O$ (260.72) calcd; C, 64.49; H, 5.03; Cl, 13.60; N, 10.74; Found: C, 64.52; H, 5.14; Cl, 13.58; N, 10.74.

2-Amino-3-chloro-N-(4-chlorobenzyl)benzamide 6c

6c was prepared according to GP3, method A or method B (4min, 300W), afford **6a** as beige powder; yield (98[a] ,80[b]%); m.p. 140°C; IR ν_{max} (KBr) 3402.46, 3328.61, 3258.44 , 1620.99cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 8.50 (1H, br.s, NH), 7.94 (1H, d, *J* = 8. 0Hz, Ph), 7.57 (1H, d, *J* = 8. 0Hz, Ph), 7.39 (2H, d, *J* = 8. 4Hz, Ph), 7.33 (2H, d, *J* = 8. 4Hz, Ph), 6.60 (1H, t, *J*= 8.0Hz, Ph), 6.56 (2H, br.s, NH_2), 4.42 (2H, d, *J*=5.8Hz, CH_2);^{13}C-NMR: δ 40.2,

115.7, 116.8, 119.62, 127.7, 128.8, 129.6, 131.8, 132.4, 139.2, 145.8, 168.7; MS *m/z* (%):295 [M^+] ($C_{14}H_{12}Cl_2N_2O$]; Anal. for $C_{14}H_{12}Cl_2N_2O$ (295.16) calcd; C, 56.97; H, 4.10; Cl, 24.02; N, 9.49; Found: C, 57.00; H, 4.13; Cl, 24.12; N, 9.48.

2-(4-(2-Amino-3-chlorobenzamido) phenyl) acetic acid 6d

6d was prepared according to GP3, method A or method B (5min, 280W), afford **6a** as beige powder; yield (60[a] ,65[b]%); m.p. 206°C; IR ν_{max} (KBr) 3466.01, 3115.82, 1763.32, 1697.62 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 12.31 (1H, s, OH), 10.18 (1H, br.s, NH), 7.63 (3H, m , Ph), 7.44 (1H, d, J = 8.0Hz, Ph), 7.23 (2H, d, J = 8.0Hz, Ph), 6.67 (1H, t, J = 8. 0Hz, Ph), 6.39 (2H, br.s, NH_2), 3.54 (2H, s, CH_2);^{13}C-NMR: δ 40.2, 115.9, 118.1, 119.5, 121.2, 128.3, 130.1, 131.0, 132.6, 138.0, 145.6, 167.5, 173.3; MS *m/z* (%):304 [M^+] ($C_{15}H_{13}ClN_2O_3$) (23.44), 289 (9.37) , 245 (12.50) , 235 (31.25),179 (100),163 (58.46); Anal. for $C_{15}H_{13}ClN_2O_3$ (304.73) calcd; C, 59.12; H, 4.30; Cl, 11.63; N, 9.19; Found: C C, 59.10; H, 4.31; Cl, 11.61; N, 9.20.

2-Amino-3-chloro-N-(4-methoxyphenyl)benzamide 6e

6e was prepared according to GP3, method A or method B (4min, 280W), afford **6e** as beige powder; yield (82[a] ,60[b]%); m.p. 176°C; IR ν_{max} (KBr) 3486.54, 3381.63 , 3311.53 , 1612.24 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 7.63 (1H, br.s, NH), 7.45(2H, d, J = 8.4Hz, Ph), 7.38-7.40 (2H, m, Ph), 6.91 (2H, d, J = 8.4Hz, Ph), 6.64 (1H, t, J = 8.0Hz, Ph), 6.02 (2H, br.s, NH_2), 3.83 (3H, s, OCH_3) ;^{13}C-NMR: δ 55.6, 114.4, 117.4, 121.1, 122.7, 122.8, 130.5, 132.5, 132.5, 145.3, 157.0, 167.0; MS *m/z* (%):277[M^+]($C_{14}H_{13}ClN_2O_2$)(26.60), 276[M^+-1](3.20), 275 [M^+-2H] (27.52) , 246 (1.33), 242 (64.12) , 240 (100); Anal. for $C_{14}H_{13}ClN_2O_2$ (276.72) calcd; C, 60.77; H, 4.74; Cl, 12.81; N, 10.12; Found: C, 60.74; H, 4.74; Cl, 12.80; N, 10.11.

2-Amino-3-chloro-N-(3,4-dimethoxyphenyl)benzamide 6f

6f was prepared according to GP3, method A or method B (4min, 140W), afford **6f** as dark gray powder; yield (95[a] ,93[b]%); m.p. 176°C; IR ν_{max} (KBr) 3449.58, 3376.05, 3323.65 , 1644.36 cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): 10.06 (1H, br.s, NH), 7.62 (1H, d, J = 8.3Hz, Ph), 7.40-7.44 (2H, m, Ph), 7.27 (1H, d, J= 8.3Hz, Ph), 6.92 (1H, d, J = 8.8Hz, Ph), 6.66 (1H, t, J = 8.3Hz, Ph), 6.39 (2H, br.s, NH_2), 3.47,3.75(6H, s, $2OCH_3$) ;^{13}C-NMR: δ 55.9, 56.3, 106.4, 112.3, 113.2, 115.9, 118.2, 119.5, 128.2, 132.5, 132.9, 145.5, 145.8, 148.9, 167.2; MS *m/z* (%):306 [M^+] ($C_{15}H_{15}ClN_2O_3$) (100), 305 [M^+-1] (90.22), 304 [M^+-2] (30.11), 307 [M^++1] (92.10), 308 [M^++2] (38.41), 291 (53.10); Anal. for $C_{15}H_{15}ClN_2O_3$ (306.74) calcd; C, 58.73; H, 4.93; Cl, 11.56; N, 9.13; Found: C, 58.71; H, 4.92; Cl, 11.50; N, 9.20.

2-Amino-3-chloro-N-(3,4-dimethoxyphenethyl)benzamide 6g

6g was prepared according to GP3, method A or method B (8min, 140W), afford **6g** as yellow powder; yield (99[a] ,60[b]%); m.p. 108°C; IR ν_{max} (KBr) 3419.05, 3355.39, 3327.86 , 1647.33cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6) (ppm): δ 8.46 (1H, br.s, NH), 6.49 (2H, br.s, NH_2) , 7.44 (1H, d, J= 7.8Hz, Ph), 7.36 (1H, d, J= 7.8Hz, Ph), 6.86 (1H, d, J= 8.0Hz, Ph), 6.82 (1H, s, Ph), 6.75(1H,d, J= 8.0Hz, Ph), 6.57(1H,t , J= 7.8 Hz, Ph), 3.71,3.72(6H, s, $2(OCH_3)$) , 3.43(2H, q, J= 6.6Hz, CH_2), 2.77 (2H, t, J= 7.3Hz, CH_2);^{13}C-NMR: δ 55.9, 56.0, 35.9, 40.5, 112.4, 113.1, 115.7, 117.6, 119.5, 121.0, 127.6, 132.1, 132.5, 145.6, 147.8, 149.1, 168.6; MS *m/z* (%):334 [M^+] ($C_{17}H_{19}ClN_2O_3$) (30.50), 335[M^++1] (100), 307 (78.92),306 (83.45) ,246 (86.00) , 228 (77.90); Anal. for $C_{17}H_{19}ClN_2O_3$ (334.80) calcd; C, 60.99; H, 5.72; Cl, 10.59; N, 8.37; Found: C, 61.03; H, 5.71; Cl, 10.61; N, 8.40.

Antifungal activity

Tested samples were screened separately in vitro for their antifungal activity various fungi viz. Aspergillus fumigatus (RCMB 002003), Geotrichum candidum (RCMB 052006) candida albicans (RCMB 005002) and syncephalastrum racemosum (RCMB 005003). The cluture of fungi was purified by single spore isolation technique. The antifungal activity was by agar well diffusion method by the following procedure:

Sabourad dextrose agar plates: A homogeneous mixture of glucose -peptone-agar (40:10:15(was sterilized by autoclaving at 121 °C for 20 min. the sterilized solution (25mL) was poured in each sterilized petridish in laminar flow and left for 20 min to form the solidified sabourad dextrose agar plate. these plates were inverted and kept at 30 °C in incubator to remove the moisture and to check for the contamination.

Antifungal Assay

Fungal strain was grown in 5 ml sabourad dextrose broth (glucose: peptone; 40:10) for 3-4 days to achieve 105CFU/mL cells. The fungal culture (0.1mL) was spread out uniformly on the sabourad dextrose agar plates by sterilized triangular folded glass rod. Plates were left for 5-10 min so that culture is properly adsorbed on the surface of sabourad dextrose agar plates. Now small wells of size (4mmx2mm) were cut into the plates with the help of well cutter and bottom of the wells were sealed with 0.8% soft agar to prevent the flow of test sample at the bottom of the well. 100 µL of the tested samples (10mg/mL) were loaded into the wells of the plates. All compounds was prepared in dimethyl sulfoxide, DMSO was loaded as control. The plates were kept for incubation at 30 °C for 3-4 days and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each fungus. clotrimaole and itraconazole were used as antifungal standard drugs.

Antibacterial activity:

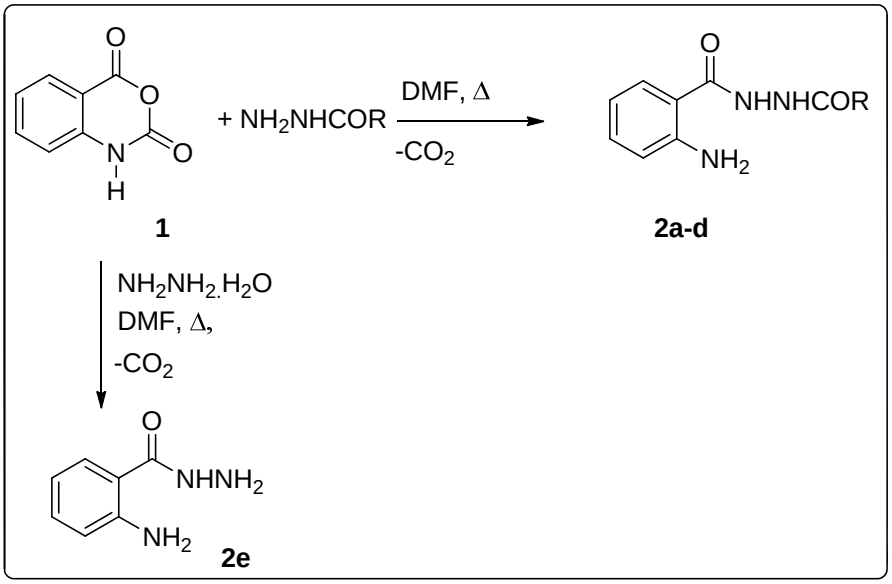
Antibacterial activities were investigated using agar well diffusion method. The activity of tested samples was studied against the Staphylococcus aureus (RCMB 000106) and Bacillis Subtilis (RCMB 000107) {as Gram positive bacteria} and pseudomonas aeruginosa (RCMB 000102) and Escherichia coli (RCMB 000103) {as gram negative bacteria }. The solution of 5 mg/ml of each compound in DMSO was prepared for testing against bacteria. Centrifuged pellets of bacteria from 24h old culture containing approximately 104-106 CFU (colony forming unit) per ml were spread on the surface of nutrient agar (typetone 1%, yeast extract 0.5%, NaCl 0.5%, agar 1000mL of distilled water, PH 7.0) which was autoclaved under 121°C for at least 20 min. Wells were created in medium with the help of sterile metallic bores and then cooled down to 45 °C. The activity was determined by measuring the diameter of the inhibition zone (in mm). 100µL of the tested samples (10mg/mL) were loaded into the wells of the plates. All compounds was prepared in DMSO, DMSO was loaded as control. The plates were kept for incubation at 37 °C for 24h and then the plates were examined for the formation of zone of inhibition. Each inhibition zone was measured three times by caliper to get an average value. The test was performed three times for each bacteria. Streptomycin was used as antibacterial standard drug.

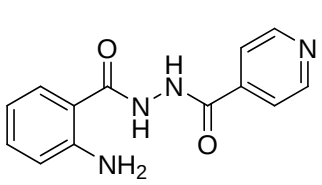
3. Results and discussion

Chemistry

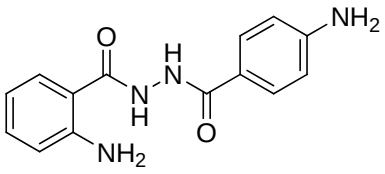
Treatment of isatoic anhydride **1** with *N*-nucleophiles, such as isonicotinohydrazide, 4-aminobenzohydrazide, 4-chlorobenzohydrazide, benzohydrazide and hydrazine hydrate in DMF under reflux condition afforded **2a-e** in good yield as depicted in Scheme 1. It is assumed that the product **2a-e** was formed *via* initial nucleophilic addition of hydrazine derivatives into carbonyl group followed ring opening and elimination of CO_2 molecules to give the desired product **2a-e**. Alternatively, **2a,e** can be obtained by microwave mediated methodology. The structures suggested for all new compounds **2a-e** are in good agreement with their analytical and spectroscopic data. IR spectra of **2a-e** exhibited a carbonyl absorption bands in the region 1695-1606 cm^{-1} in addition to the absorption bands of two NH functions in the region. Their ^1H NMR showed two D_2O exchangeable singlet signals assignable for two NH groups. The mass spectra of compounds confirmed the assigned structures.

Scheme 1. Synthesis of 2-amino-N -(substituted benzoyl)substituted hydrazide **2a-e** isatoic anhydride **1**.

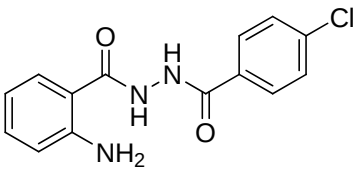




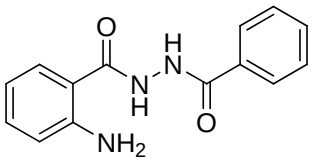
2a



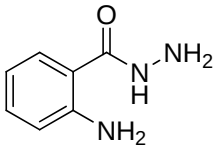
2b



2c



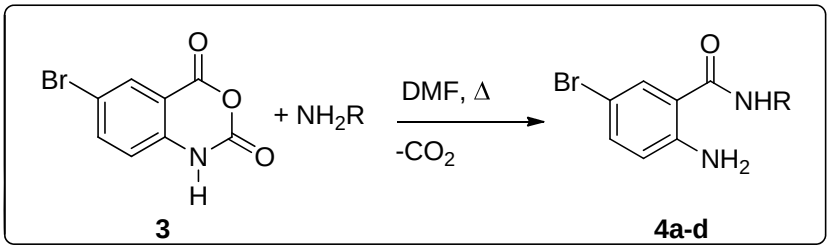
2d



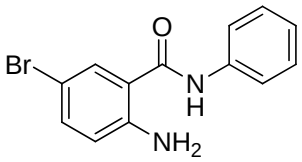
2e

The synthesis of the amides **4a-d** were carried out by reacting 8-bromo isatoic anhydride **3** with different amine for example (3,4-dimethoxyphenyl)methanamine, 3,4-dimethoxyaniline, 4-chloroaniline and aniline in DFM under reflux for 6h afford the products in very good yield as depicted in scheme 2. Alternatively, **4c,d** can be obtained by microwave mediated methodology. The structures of these compounds were determined by ^{1}H-NMR, EI, IR and UV spectroscopic, and micro analyses for carbon, hydrogen and nitrogen.

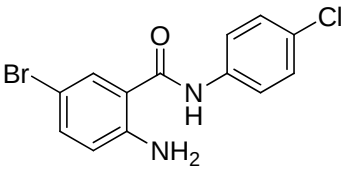
Scheme 2. Synthesis of 2-Amino-N-substituted-5-bromo benzamide **4a-d** from 8-bromoisatoic anhydride



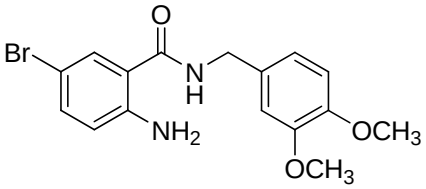
3 **4a-d**



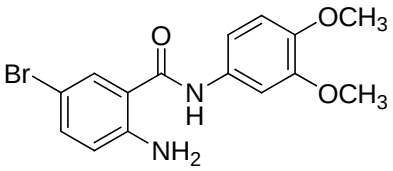
4a



4b



4c

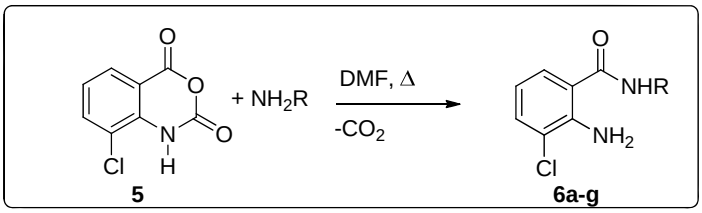


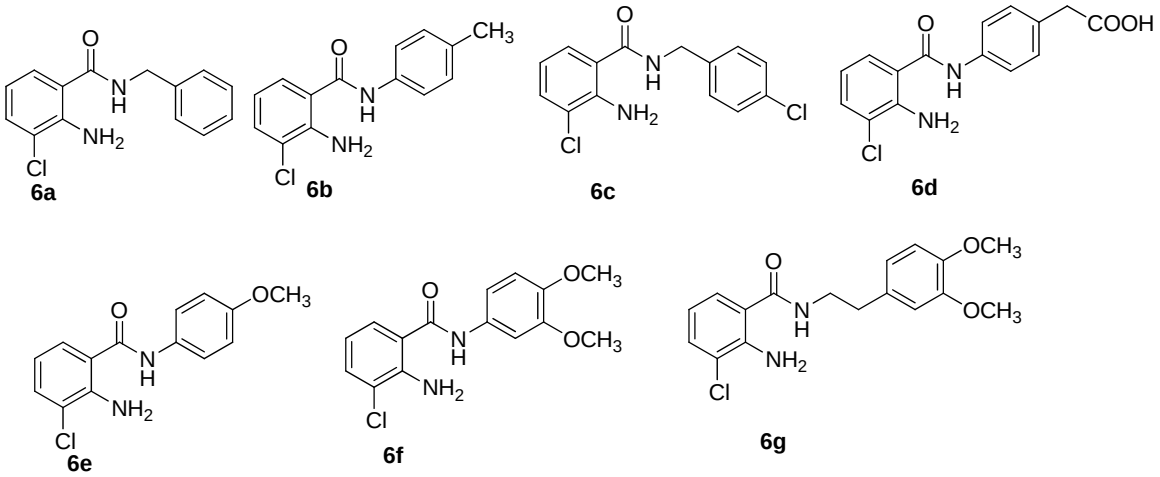
4d

Next, refluxing of 10-chloroisatoic anhydride **5** with different amines derivatives such as benzyl amine, toulidine, p-chlorobenzylamine, anisidine, 3-mehtoxyanisidine, 2-(3,4-dimethoxyphenyl)ethanamine, 2-(4-aminophenyl)acetic acid afforded **6a-g** in very good yield as drawn in scheme 3.The Structures of **6a-g** were established through, spectroscopic and elemental analysis data. Is noteworthy to mention that **6c-g** can be synthesized using microwave method in good yields.

It is assumed that the products **4a-d and 6a-g** were formed *via* initial nucleophilic addition of amine derivatives into carbonyl group followed ring opening and elimination of CO_2 molecules to give the desired product.

Scheme 3. Synthesis of 2-Amino-N-substituted-5-chloro benzamide **6a-g** from 10-chloroisatoic anhydride





Antimicrobial evaluation

Antifungal activity

Some of the synthesized compounds **2a-c,e,j, 4a-c,** and **6d,e** were tested for their antifungal activity against Aspergillus fumigatus (RCMB 002003), Geotrichum candidum (RCMB 052006) candida albicans (RCMB 005002) and syncephalastrum racemosum (RCMB 005003). Clotrimazole was used as standard antifungal agent. The result are shown in Table 1 displayed that compounds **3b** exhibited excellent antifungal activity against all the two strains of fungi (zones of inhibition range from 18 to 28 mm) compared to standard drug. This compound 3b (26.1 ± 0.5mm) found to be more potent than Clotrimazole (18.3 ± 0.6mm) against Aspergillus fumigatus. Compound **4b** (**23.1 ± 0.4**mm) showed potent against Saccharomyces cerevisiae and found to be the same active as standard Clotrimazole (**23.1 ± 0.4**mm), fruther more, **4b** (18.3 ± 0.6mm) showed potent against C. albicans and found to be less active than standard Clotrimazole (26.1 ± 0.5mm). All other compounds found to be less effective.

Antibacterial activity

Antibacterial activity of some of the synthesized compounds 2a-**c,e,j, 4a-c,** and **6d,e** were tested using agar well diffusion method [22]. The activity of the tested compounds was studied against the Bacillus subtilis (ATCC6633), Staphylococcus aureus (ATCC29213) (Gram positive bacteria) and Escherichia coli (ATCC25922), Pseudomonas aeruginosa (ATCC27953) (Gram negative bacteria). Standard antibiotic, Streptomycin was used as standard antibacterial agent specified in US pharmacopeia at (25µg/ ml).

The results of preliminary antibacterial testing of compounds are, shown in Table 1, revealed that, Compound **4b** display excellent activity against Gram positive bacteria and Gram negative bacteria (zones of inhibition range from 24 to 30 mm). This compound found to be more potent than streptomycin against B. subcilils and. Compound **4b** (30.1 ± 0.6mm) showed potent against B. subcilils and found to be more active than standard streptomycin (24.3 ± 0.4mm), furthermore, **3b** (25.6 ± 0.04mm) showed potent against Escherichia coli and found to be more active than standard streptomycin (25.1 ± 0.5 mm) too. On the other hand it revealed excellent activity against Staphylococcus aureus, and Pseudomonas aeruginosa with zone inhibition 25.1 ± 0.5mm, and 24.3 ± 0.08mm respectively while the standard streptomycin is 25.6 ± 0.5 mm, and 30.1 ± 0.6 mm respectively. All other compounds found to be less effective.

Table 1. Antimicrobial activity of the newly synthesized compounds.

Comp. No.	GRAM-POSTIVE BACTERIA		GRAM-NEGATIVE BACTERIA		FUNGI		
	STAPHYLOCOCCUS AUREUS	***BACILILS SUBTILIS***	***PSEUDOMONAS AERUGINOSA***	***ESCHERICHIA COLI***	***ASPERGILLUS FUMIGATUS***	***SACCHAROMYCES CEREVISIAE***	***CANDIDA ALBICANS***
2_a	8.3 ± 0.3	9.6 ± 0.5	9.1 ± 0.3	9.8 ± 0.3	11.4 ± 0.4	9.7 ± 0.3	8.1 ± 0.3
2_b	10.6 ± 0.3	10.8 ± 0.4	10.9 ± 0.4	11.2 ± 0.3	11.8 ± 0.3	12.6 ± 0.4	11.5 ± 0.5
2_c	10.4 ± 0.3	11.2 ± 0.3	9.8 ± 0.4	10.2 ± 0.4	11.1 ± 0.4	9.4 ± 0.3	11.1 ± 0.2
2_e	10.9 ± 0.4	11.5 ± 0.3	10.3 ± 0.4	10.8 ± 0.3	11.1 ± 0.4	9.8 ± 0.3	10.4 ± 0.5
2_j	12.2 ± 0.3	11.1 ± 0.4	10.7 ± 0.3	10.1 ± 0.4	11.9 ± 0.4	12.5 ± 0.4	13.4 ± 0.4
4b	25.1 ± 0.5	30.1 ± 0.6	24.3 ± 0.08	25.6 ± 0.04	26.1 ± 0.5	23.1 ± 0.4	18.3 ± 0.6
4c	11.2 ± 0.4	10.8 ± 0.4	9.7 ± 0.2	8.9 ± 0.4	12.1 ± 0.5	11.9 ± 0.4	11.7 ± 0.5
6d	9.9 ± 0.4	10.3 ± 0.4	10.1 ± 0.3	9.7 ± 0.3	10.5 ± 0.5	11.2 ± 0.4	10.9 ± 0.3
6e	9.3 ± 0.4	10.1 ± 0.4	9.7 ± 0.3	9.1 ± 0.2	9.8 ± 0.3	8.1 ± 0.3	8.4 ± 0.2
CLOTRIMAZOLE	-	-	-	-	**18.3 ± 0.6**	**23.1 ± 0.4**	**26.1± 0.5**
STREPTOMYCIN	**25.6 ± 0.5**	**24.3 ± 0.4**	**30.1 ± 0.6**	**25.1 ± 0.5**	-	-	-
Inhibition zones (mm)							

4. Conclusions

From the obtained results, we can summarized that we have successfully prepared series of hydrazides derivatives **2a-e** starting from isatoic anhydride **1** with appropriate *N*-nucleophile assisted by microwave or classical methods. Amides **4a-d** and **6a-g** have been synthesized from isatoic anhydride derivatives **3** and **5**. The antibacterial and antifungal activities of some of the synthesized compounds were evaluated. **4b** exhibited the highest antibacterial and antifungal activities comparable to standard antibiotics.

Acknowledgments

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