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Highly Regioselective Ring-Opening of Epoxides: Synthesis and Biological Evaluation as Potent Antimicrobial Agents

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ABSTRACT

In present work, a convenient method for the nucleophilic ring-opening of epoxides with secondary amine in presence of ethyl acetate as a polar aprotic solvent using catalytic amount of base is described. Present method is highly regioselective and furnishes the products in short time of period with excellent yield. The regioselectivity of this ring opening was confirmed using FT-IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectral data. The antimicrobial screening of all these synthesized compounds was done against some bacterial and fungal strains in two polar solvents, DMSO and DMF using agar well diffusion method. These compounds showed good inhibition of bacterial strains and potent against fungal strains than standard drug.

KEYWORDS

Benzohydrazides, Regioselctivity, Ring-opening, Antimicrobial activity.

INTRODUCTION

The effectiveness of antibiotic treatment is under absolute threat due to the rampant proliferation of resistant pathogens. The ability of these resistant strains to be effective against antibiotics currently poses a serious threat to the lives of patients. This phenomenon of resistance raises great concerns about the discovery of new targets and drugs in chemotherapy [1]. Hydrazones containing an azometine -NHN=CH- group are synthesized by heating the different substituted hydrazines/ hydrazides with appropriate aldehydes and ketones using solvents such as ethanol, methanol, butanol, tetrahydrofuran, glacial acetic acid. Hydrazide-hydrazones compounds are very important intermediates and they are highly effective organic compounds individually. When they are used as intermediates, coupling products can be produced by using the active hydrogen component of –CONHN=CH- azometine group. Literature survey revealed that the azomethine moiety of hydrazide–hydrazone compounds has significant role as antitumor agent [2,3]. Newly synthesized hydrazide derivatives were potent against human colorectal (HCT116) and MGC803 cancer cells lines [4]. It has recently been reported that a hydrazide compounds are highly effective and selective inhibitors of antibacterial activity [5] against fungus [6], anti-inflammatory [7], antioxidant [8], antimalarial activity [9], anticonvulsant

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[10], analgesic [11], antiplatelets [12], antituberculosis [13], antiplasmodial and antileishmanial activity [14]. Some benzohydrazides are significant and potent agents against various cancer cell lines such as A549, MCF7, HeLa [15]. Various studies are on-going for the development of specific target-based drugs to minimize toxicity, drug resistance, dosage [16] and improve their activity with lower drawbacks [17].

All these facts encouraged us to integrate this new series of benzohydrazide derivatives are formed by highly regio-selective ring-opening of epoxide with diethylamine and screened these new derivatives as potential antimicrobial agents. The chemical structure of benzohydrazides was confirmed by various spectroscopic techniques such as FT-IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The antimicrobial screening was carried out using various strains including Gram-positive bacterial: *Staphylococcus aureus* (MTCC 9886), *Bacillus subtilis* (MTCC 441) and *Micrococcus luteus* (MTCC 9278), Gram-negative bacteria: *Escherichia coli* (MTCC 1652), *Pseudomonas aeruginosa* MTCC 741 and *Salmonella typhi* MTCC 98 and fungal strains: *Saccharomyces cerevisiae* MTCC 170 and fungi *Aspergillus niger* MTCC 282 and *Penicillium chrysogenum* MTCC 5108 by agar well diffusion method.

EXPERIMENTAL

All the analytical grade solvents, chemicals and reagents were purchased from commercial sources and used without further purification. Substituted benzaldehydes, hydrazine hydrate, 4-methoxybenzaldehyde, isopropanol, ethanol 95%, chloroform, acetone, ethyl acetate, *n*-hexane were purchased from Spectrochem. The formation of compounds and it's purity was cheked by thin layer chromatography-TLC (performed on aluminium coated TLC plates gel-G60 F_{254}. The melting points of all derivatives were determined by open capillary method and are uncorrected. Infrared spectra were recorded on KBr discs, FT-IR instrument (Shimadzu spectrophotometer Model 8400). ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) were recorded on a Bruker instrument in DMSO-d_6 using TMS as a standard solvent. A GCMSQP2010 mass spectrometer (Shimadzu) was used to resolve the mass spectra of the synthesized analogues.

General procedure for the synthesis of 4-hydroxybenzohydrazide: A mixture of methyl paraben (0.1 mol) and hydrazine hydrate (99%) (3 mol) was refluxed with hydrazine hydrate for 12-14 h. The progress of reaction was monitored by TLC. The reaction mixture was cooled to room temperature and then poured into crushed ice and filtered. Obtained precipitates were recrystallized with absolute ethanol. The purity was checked by thin layer chromatography (TLC) (performed on aluminium coated plates Gel 60 F_{254} (E. Merck) using MeOH-MDC (1:9) mobile phase.

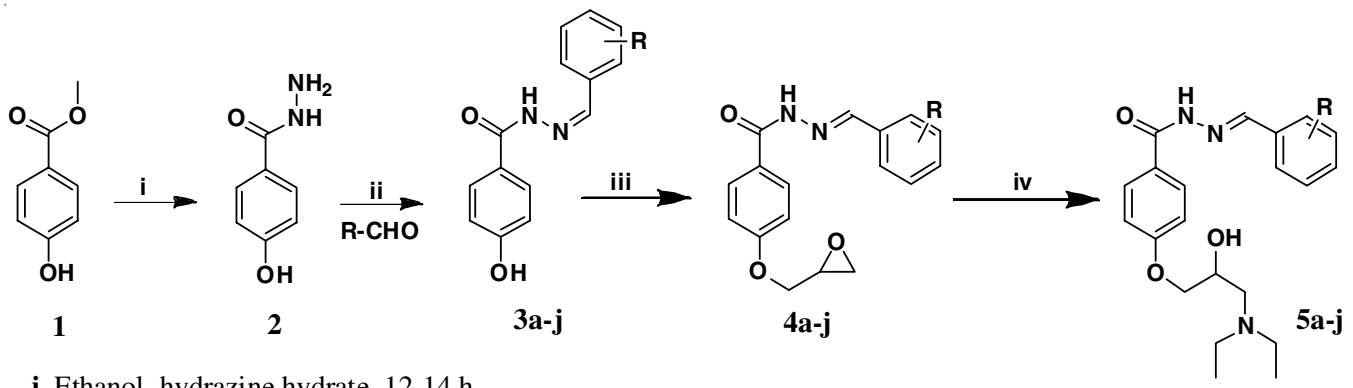
Synthesis of substituted *N′*-benzylidene-4-hydroxybenzohydrazide (3a-j): A mixture of 4-hydroxybenzohydrazide (0.5 g, 0.003 mol) and substituted benzaldehyde (0.003 mol) was refluxed for 2 h in the presence of glacial acetic acid in a catalytic amount in ethanol. Completion of the reaction was confirmed by thin-layer chromatography (TLC). After that, the reaction mixture was poured into 500 mL beaker containing ice-cold water with continous stirring. The obtained solid was then filtered and dried under vacuum to obtain crude product (**3a-j**). The purity of crude product was checked by TLC using ethyl actate:hexane (2:8) eluent.

Synthesis of substituted *N′*-benzylidene-4-(oxiran-2-ylmethoxy)benzohydrazide derivatives (4a-j): A mixture of substituted *N′*-benzylidene-4-hydroxybenzohydrazide (0.003 mol), epicholorohydrin (0.014 mol) and potassium *t*-butoxide (0.004 mol) in DMF stirred at room temperature for 3-4 h. The completion of reaction was confirmed by thin layer chromatography (TLC) using (ethyl acetate: toluene 4:6) as mobile phase. After completion of reaction, the reaction mixture was poured into ice cold water and the resulting solid was filtered, washed with ethyl acetate:hexane (2:8) to remove other impurities and dried under vacuum to give crude product. The obtained crude product was purified by recrystallization with isopropanol, all these compounds (**4a-j**) was ascertained by TLC.

Synthesis of substituted *N′*-benzylidene-4-(3-(diethylamino)-2-hydroxypropoxy)benzohydrazide (5a-j): A mixture of substituted *N′*-benzylidene-4-(oxiran-2-ylmethoxy)benzohydrazide (0.003 mol), diethylamine (0.003 mol) and catalytic amount of NaOH was refluxed in ethyl acetate for 2 h. Progress of the reaction was observed by thin layer chromatography (TLC) using (8:2 MDC:methanol) as mobile phase. Then, the reaction mixture was cooled to room temperature and then added ice cold water. The resulting solid was filtered, washed and dried under vacuum to give crude product. The obtained crude product was confirmed by staining reagent DNP by TLC and finally recrystallized in ethanol (**Scheme-I**).

***N′*-(4-Methoxybenzylidene)-4-(oxiran-2-ylmethoxy)-benzohydrazide (4a):** White solid; yield: 89%; R_f value: 0.41 (ethyl acetate:toluene, 4:6); m.p.: 102-104 ºC; IR (KBr, ν_{max}, cm^{-1}): 3178 (-NH (amide)sym. *str.*), 3032 (Ar-H *str.*), 2839, 2553 (-CH_2. asym. and sym. *str.*), 1898 (C-H *bend*. overtone), 1643, 1604, (C=O (amides) *str.*), 1550, 1357 (C=C *str.* phenyl nucleus), 1257 (asym. Ar- C-O bond *str.*), 1172 (asym. aliphatic C-O *str.*), 1026 (sym. Ar- C-O *str.*) 918 (epoxy ring vibrations), 840, 763 (*p*-disubstituted phenyl ring), 676 (C-C out of plane *bend.*). ^{1}H NMR (400 MHz, DMSO) in δ ppm: 2.737 (quartet, 1H of oxirane ring), 2.869 (triplet, 1H of oxirane ring), 3.63 (quartet, 1H of oxirane ring), 3.811 (s, 3H of –CH_3), 3.917 (quartet, 1H of CH_2), 4.435 (dd, 1H of CH_2), 7.0625 (dd, 4H, aromatic), 7.668 (d, 2H, aromatic), 7.9 (d, 2H, aromatic), 8.387 (s, 1H of NH), 11.62 (s, 1H of benzylidenimin); MS (*m/z*): 325.8 (M); Elemental anal. calcd. (found) % for $C_{18}H_{18}N_2O_4$; C, 66.25 (65.22); H, 5.56 (5.46); N, 8.58 (8.37); O, 19.61 (19.21).

***N′*-Benzylidene-4-(oxiran-2-ylmethoxy)benzohydrazide (4b):** White solid; yield: 86%; R_f value: 0.46 (ethyl acetate: toluene, 4:6); m.p.: 110-112 ºC; IR (KBr, ν_{max}, cm^{-1}): 3171 (-NH (amide)sym. *str.*), 3039 (Ar-H *str.*), 2916, 2607 (-CH_2. asym. and sym. *str.*), 2214 (C-H *bend*. overtone), 1651, 1612, (C=O (amides) *str.*), 1550, 1450, 1357 (C=C *str.* phenyl nucleus), 1257 (asym. Ar- C-O bond *str.*), 1172 (asym. aliphatic C-O *str.*), 1033 (sym. Ar- C-O *str.*) 949, 918 (epoxy ring vibrations), 849, 763 (*p*-disubstituted phenyl ring), 686 (C-C out of plane *bend.*); ^{1}H NMR (400 MHz, DMSO) in δ ppm: 2.739 (quartet, 1H of oxirane ring), 2.870 (triplet, 1H of oxirane ring), 3.346-3.385 (multiplet, 1H of oxirane ring), 3.923 (quartet, 1H of CH_2), 4.441 (dd, 1H of CH_2), 7.1 (d, 2H, aromatic), 7.450



i. Ethanol, hydrazine hydrate, 12-14 h
ii. Ethanol, Gla. CH_3COOH, reflux, 2 h
iii. Dimethylformamide, $(CH_3)_3COK$, rt, 3-4 h
iv. Ethylacetate, diethylamine, NaOH, reflux, 2 h

R = **a** = C_7H_8O; **b** = C_6H_5; **c** = $C_8H_{10}O$;
d = C_6H_4Br; **e** = $C_6H_4NO_2$; **f** = C_6H_4Cl;
g = C_6H_4F; **h** = C_6H_4Cl; **i** = C_4H_3S; **j** = C_5H_5N

Scheme-I: Synthetic route of substituted *N*′-benzylidene-4-(3-(diethylamino)-2-hydroxypropoxy)benzohydrazide

(triplet, 3H, aromatic), 7.728 (d, 2H, aromatic), 7.917 (d, 2H, aromatic), 8.454 (s, 1H of NH), 11.755 (s, 1H of benzylidenimine); MS (*m/z*): 295.9 (M); Elemental anal. calcd. (found) % for $C_{17}H_{16}N_2O_3$: C, 68.91 (67.22); H, 5.44 (5.41); N, 9.45 (9.36); O, 16.20 (15.21).

4-(3-(Diethylamino)-2-hydroxypropoxy)-*N*′-(4-methoxybenzylidene)benzohydrazide (5a): Off white solid; Yield: 89%; R_f value: 0.35 (methanol:MDC, 8:2); m.p.: 120-122 ºC; IR (KBr, ν_{max}, cm^{-1}): 3540 (-OH (alcohol) sym. *str.*), 3156 (-NH (amide) sym. *str.*), 3024 (Ar-H *str.*), 2839, 2533 (-CH_2 asym. and sym. *str.*), 1633, 1614 (C=O (amides) *str.*), 1535, 1337 (C=C *str.* phenyl nucleus), 1263 (asym. Ar-C-O bond *str.*), 1162 (asym. aliphatic C-O *str.*), 1014 (sym. Ar-C-O *str.*), 843, 733 (*p*-disubstituted phenyl ring), 671 (C-C out of plane bending). ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.958 (m, 8H, aliphatic), 3.884-4.101 (m, 6H, aliphatic), 4.836 (d, 1H of OH), 7.033-8.571 (m, 12H, aromatic), 8.845 (s, 1H of NH), 11.739 (s, 1H of benzylidenimine); MS (*m/z*): 398.8 (M); Elemental anal. calcd. (found) % for $C_{22}H_{29}N_3O_4$: C, 66.14 (65.12); H, 7.32 (6.86); N, 10.52 (9.33); O, 16.02 (15.21).

***N*′-Benzylidene-4-(3-(diethylamino)-2-hydroxypropoxy)benzohydrazide (5b):** White solid; yield: 86%; R_f value: 0.31 (methanol:MDC, 8:2); m.p.: 122-124 °C; IR (KBr, ν_{max}, cm^{-1}): 3550 (-OH (alcohol) sym. *str.*), 3175 (-NH (amide) sym. *str.*), 3030 (Ar-H *str.*), 2829, 2543 (-CH_2. asym. and sym. *str.*), 1643, 1604 (C=O (amides) *str.*), 1545, 1347 (C=C *str.* phenyl nucleus), 1253 (asym. Ar- C-O bond *str.*), 1172 (asym. aliphatic C-O *str.*), 1024 (sym. Ar- C-O *str.*), 840, 763 (*p*-disubstituted phenyl ring), 675 (C-C out of plane bending). ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.960 (m, 8H, aliphatic), 3.881-4.106 (m, 4H, aliphatic), 4.834 (d, 1H of OH), 7.031-8.569 (m, 13H, aromatic), 8.835 (s, 1H of NH), 11.736 (s, 1H of benzylidenimine); MS (*m/z*): 368.9 (M); Elemental anal. calcd. (found) % for $C_{21}H_{27}N_3O_3$: C, 68.27 (67.12); H, 7.37 (6.96); N, 11.37 (11.33); O, 12.99 (11.21).

4-(3-(Diethylamino)-2-hydroxypropoxy)-*N*′-(2,5-dimethoxybenzylidene)benzohydrazide (5c): Yellowish white solid; yield: 85 %; R_f value: 0.29 (methanol:MDC, 8:2); m.p.: 103-105 ºC; IR (KBr, ν_{max}, cm^{-1}): 3540 (-OH (alcohol) sym. *str.*), 3174 (-NH (amide) sym. *str.*), 3024 (Ar-H *str.*), 2824, 2540 (-CH_2. asym. and sym. *str.*), 1640, 1624 (C=O (amides) *str.*), 1549, 1367 (C=C *str.* phenyl nucleus), 1248 (asym. Ar-C-O bond *str.*), 1170 (asym. aliphatic C-O *str.*), 1022 (sym. Ar-C-O *str.*), 849, 762 (*p*-disubstituted phenyl ring), 673 (C-C out of plane bending). ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.964 (m, 8H, aliphatic), 3.851-4.102 (m, 9H, aliphatic), 4.828 (d, 1H of OH), 7.011-8.549 (m, 11H, aromatic), 8.841 (s, 1H of NH), 11.745 (s, 1H of benzylidenimine); MS (*m/z*): 428.8 (M); Elemental anal. calcd. (found) % for $C_{23}H_{31}N_3O_5$: C, 64.32 (64.12); H, 7.27 (6.56); N, 9.78 (8.03); O, 18.63 (17.21).

***N*′-(4-Bromobenzylidene)-4-(3-(diethylamino)-2-hydroxypropoxy)benzohydrazide (5d):** White solid; yield: 86%; R_f value: 0.39 (methanol:MDC, 8:2); m.p.: 122-124 ºC; IR (KBr, ν_{max}, cm^{-1}): 3530 (-OH (alcohol) sym. *str.*), 3167 (-NH (amide) sym. *str.*), 3029 (Ar-H *str.*), 2819, 2548 (-CH_2. asym. and sym. *str.*), 1641, 1634 (C=O (amides) *str.*), 1535, 1377 (C=C *str.* phenyl nucleus), 1263 (asym. Ar-C-O bond *str.*), 1168 (asym. aliphatic C-O *str.*), 1014 (sym. Ar-C-O *str.*), 830, 760 (*p*-disubstituted phenyl ring), 670 (C-C out of plane bending), 667 (C-Br *str.*). ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.959 (m, 10H, aliphatic), 3.879-4.104 (m, 4H, aliphatic), 4.832 (d, 1H of OH), 7.030-8.568 (m, 9H, aromatic), 8.832 (s, 1H of NH), 11.733 (s, 1H of benzylidenimine); MS (*m/z*): 447.9 (M); Elemental anal. calcd. (found) % for $C_{21}H_{26}BrN_3O_3$: C, 56.26 (55.22); H, 5.85 (4.96); N, 9.37 (8.33); O, 10.71 (9.81).

4-(3-(Diethylamino)-2-hydroxypropoxy)-*N*′-(3-nitrobenzylidene)benzohydrazide (5e): Yellowish solid; yield: 76%; R_f value: 0.29 (methanol:MDC, 8:2); m.p.: 134-136 ºC; IR (KBr, ν_{max}, cm^{-1}): 3580 (-OH (alcohol) sym. *str.*), 3171 (-NH (amide) sym. *str.*), 3032 (Ar-H *str.*), 2823, 2602 (-CH_2. asym. and sym. *str.*), 1644, 1656 (C=O (amides) *str.*), 1598 (nitro *str.*), 1585, 1349 (C=C *str.* phenyl nucleus), 1303 (C-NO_2 *str.*), 1263 (asym. Ar- C-O bond *str.*), 1146 (asym. aliphatic C-O *str.*), 1047 (sym. Ar- C-O *str.*), 840, 762 (*p*-disubstituted phenyl ring), 684 (C-C out of plane bending); ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.947 (m, 10H, aliphatic), 3.866-4.101 (m, 4H, aliphatic), 4.821 (d, 1H of OH), 7.020-8.531 (m, 9H, aromatic), 8.816 (s, 1H of NH), 11.711 (s, 1H of benzylidenimine); MS (*m/z*): 413.8 (M); Elemental anal. calcd. (found) % for $C_{21}H_{26}N_4O_5$: C, 60.86 (59.28); H, 6.32 (6.11); N, 13.52 (13.01); O, 19.30 (18.61).

***N′*-(4-Chlorobenzylidene)-4-(3-(diethylamino)-2-hydroxypropoxy)benzohydrazide (5f):** Off white solid; yield: 90%; R_f value: 0.38 (methanol:MDC, 8:2); m.p.: 126-128 ºC; IR (KBr, ν_{max}, cm^{-1}): 3534 (-OH (alcohol) sym. *str.*), 3165 (-NH (amide) sym. *str.*), 3027 (Ar-H *str.*), 2821, 2548 (-CH_2. asym. and sym. *str.*), 1644, 1624 (C=O (amides) *str.*), 1585, 1369 (C=C *str.* phenyl nucleus), 1253 (asym. Ar- C-O bond *str.*), 1158 (asym. aliphatic C-O *str.*), 1024 (sym. Ar- C-O *str.*), 832, 761 (*p*-disubstituted phenyl ring), 690 (C-C out of plane bending), 667 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.958 (m, 10H, aliphatic), 3.876-4.101 (m, 4H, aliphatic), 4.829 (d, 1H of OH), 7.029-8.558 (m, 9H, aromatic), 8.830 (s, 1H of NH), 11.729 (s, 1H of benzylidenimine); MS (*m/z*): 403.3 (M); Elemental anal. calcd. (found) % for $C_{21}H_{26}N_3O_3Cl$: C, 62.45 (61.28); H, 6.49 (5.91); N, 10.40 (10.03); O, 11.88 (10.81).

4-(3-(Diethylamino)-2-hydroxypropoxy)-*N′*-(4-fluorobenzylidene)benzohydrazide (5g): Light yellowish solid; yield: 81 %; R_f value: 0.32 (methanol:MDC, 8:2); m.p.: 112-114 ºC; IR (KBr, ν_{max}, cm^{-1}): 3574 (-OH (alcohol) sym. *str.*), 3155 (-NH (amide) sym. *str.*), 3024 (Ar-H *str.*), 2828, 2598 (-CH_2. asym. and sym. *str.*), 1634, 1626 (C=O (amides) *str.*), 1575, 1399 (C=C *str.* phenyl nucleus), 1339 (C-F *str.*), 1263 (asym. Ar- C-O bond *str.*), 1148 (asym. aliphatic C-O *str.*), 1044 (sym. Ar- C-O *str.*), 842, 767 (*p*-disubstituted phenyl ring), 688 (C-C out of plane bending). ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.951 (m, 10H, aliphatic), 3.866-4.101 (m, 4H, aliphatic), 4.821 (d, 1H of OH), 7.023-8.551 (m, 9H, aromatic), 8.826 (s, 1H of NH), 11.721 (singlet, 1H of benzylidenimine); MS (*m/z*): 386.7 (M); Elemental anal. calcd. (found) % for $C_{21}H_{26}N_3O_3F$: C: 65.10 (64.28); H: 6.76 (6.41); N: 10.85 (10.01); O: 12.30 (11.61).

***N′*-(3-Chlorobenzylidene)-4-(3-(diethylamino)-2-hydroxypropoxy)benzohydrazide (5h):** White solid; yield: 92%; R_f value: 0.38 (methanol:MDC, 8:2); m.p.: 128-130 ºC; IR (KBr, ν_{max}, cm^{-1}): 3532 (-OH (alcohol) sym. *str.*), 3168 (-NH (amide) sym. *str.*), 3029 (Ar-H *str.*), 2826, 2550 (-CH_2. asym. and sym. *str.*), 1648, 1628 (C=O (amides) *str.*), 1588, 1371 (C=C *str.* phenyl nucleus), 1258 (asym. Ar-C-O bond *str.*), 1161 (asym. aliphatic C-O *str.*), 1028 (sym. Ar-C-O *str.*), 830, 765 (*p*-disubstituted phenyl ring), 694 (C-C out of plane bending), 671 (C-Cl *str.*). ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.959 (m, 10H, aliphatic), 3.878-4.103 (m, 4H, aliphatic), 4.830 (d, 1H of OH), 7.031-8.557 (m, 9H, aromatic), 8.832 (s, 1H of NH), 11.730 (s, 1H of benzylidenimine); MS (*m/z*): 403.4 (M); Elemental anal. calcd. (found) % for $C_{21}H_{26}N_3O_3Cl$: C, 62.45 (61.27); H, 6.49 (5.90); N, 10.40 (10.02); O, 11.88 (10.80).

4-(3-(Diethylamino)-2-hydroxypropoxy)-*N′*-(thiophen-2-ylmethylene)benzohydrazide (5i): Brown solid; yield:68%; R_f value: 0.41 (methanol:MDC, 8:2); m.p.: 108-110 ºC; IR (KBr, ν_{max}, cm^{-1}): 3539 (-OH (alcohol) sym. *str.*), 3169 (-NH (amide) sym. *str.*), 3019 (Ar-H *str.*), 2806, 2590 (-CH_2. asym. and sym. *str.*), 1638, 1628 (C=O (amides) *str.*), 1588, 1371 (C=C *str.* phenyl nucleus), 1258 (asym. Ar- C-O bond *str.*), 1161 (asym. aliphatic-O *str.*), 1028 (sym. Ar- C-O *str.*), 694 (C-C out of plane bending); ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.978 (m, 6H, aliphatic), 3.865-4.150 (m, 6H, aliphatic), 3.821 (d, 1H of OH), 4.211-4.319 (m, 3H, aliphatic), 8.802 (s, 1H of NH), 7.011-8.527 (m, 7H, aromatic), 11.700 (s, 1H of benzylidenimine); MS (*m/z*): 374.5 (M); Elemental anal. calcd. (found) % for $C_{19}H_{25}N_3O_3S$: C, 60.78 (60.17); H, 6.71 (5.93); N, 11.19 (11.02); O, 12.78 (11.76).

4-(3-(Diethylamino)-2-hydroxypropoxy)-*N'*-(pyridin-2-ylmethylene)benzohydrazide (5j): Light orange solid; yield: 78%; R_f value: 0.37 (methanol:MDC, 8:2); m.p.: 125-127 ºC; IR (KBr, ν_{max}, cm^{-1}): 3541 (-OH (alcohol) sym. *str.*), 3173 (-NH (amide) sym. *str.*), 3021 (Ar-H *str.*), 2800, 2599 (-CH_2. asym. and sym. *str.*), 1648, 1648 (C=O (amides) *str.*), 1598, 1371 (C=C *str.* phenyl nucleus), 1268 (asym. Ar-C-O bond *str.*), 1161 (asym. aliphatic -O *str.*), 1038 (sym. Ar-C-O *str.*), 644 (C-C out of plane bending); ^{1}H NMR (400 MHz, DMSO) in δ ppm: 0.998 (m, 6H, aliphatic), 3.855-4.140 (m, 6H, aliphatic), 3.819 (d, 1H of OH), 4.201-4.309 (m, 3H, aliphatic), 8.902 (s, 1H of NH), 7.019-8.517 (m, 8H, aromatic), 11.738 (s, 1H of benzylidenimine); MS (*m/z*): 369.9 (M); Elemental anal. calcd. (found) % for $C_{19}H_{26}N_4O_3$: C, 64.84 (63.27); H, 7.07 (6.90); N, 15.12 (14.19); O, 12.96 (11.86).

Biological activity: Antibacterial activity was determined against Gram-positive bacteria including *Micrococcus luteus* (MTCC 9278), *Staphylococcus aureus* (MTCC 9886) and *Bacillus subtilis* (MTCC 441); Gram-negative bacteria including *Escherichia coli* (MTCC 1652), *Salmonella typhi* (MTCC 98) and *Pseudomonas aeruginosa* (MTCC 741); Fungal strains including *Saccharomyces cerevisiae* (MTCC 170), *Aspergillus niger* (MTCC 282) and *Penicillium chrysogenum* (MTCC 5108). The biological evaluation of synthesized derivatives at various concentrations such as 250 µg/mL, 500 µg/mL and 1000 µg/mL by well diffusion method by using two polar solvents DMSO and DMF. The activity was compared with known antibiotic ciprofloxacin as standard.

RESULTS AND DISCUSSION

A reaction between 4-hydroxybenzohydrazide (1 equiv.) and substituted aldehydes (1 equiv.) in presence of glacial acetic acid (catalytic amount) in ethanol (10 mL) gave encouraging yield of 4-hydroxy-*N′*-(4-methoxybenzylidene)-benzohydrazide (**3a**) (94%). The well-synthesized and pure benzohydrazide was then adopted to form a new series of *N′*-(4-methoxybenzylidene)-4-(oxiran-2-yloxy)benzohydrazide derivatives (**4a-j**) by reaction with epicholorohydrin (4 equiv.) in DMF. On the basis of this reassuring result, reaction optimization studies were carried out to estimate the effect of different bases (entries 1-10, Table-1) in different solvents with different temperatures to get target molecule with excellent yield in short time compare to others. As the results shows, potassium *tert*-butoxide (1.5 equiv.) was found to be most effective base, whereas potassium carbonate gave discouraging yield (27%, entry 1). Additionally, caesium carbonate showed moderate efficiency to give desired product (entries 6 & 8). Further, the effect of reaction temperature on the amount of yield was evaluated, which leads to the conclusion that decreasing temperature led to less percentage of yield of product formation as well as increasing reaction temperature also not suitable condition for formation of product (entries 4, 6 & 8). It was also found that DMF is better choice solvent, while other solvents give unsatisfactory yield of products. Therefore, the optimal conditions for this reaction are the mixture of 4-hydroxy-

TABLE-1
OPTIMIZATION REACTION CONDITIONS TO SYNTHESIZE SUBSTITUTED *N*′-BENZYLIDENE-4-(OXIRAN-2-YLOXY)BENZOHYDRAZIDE DERIVATIVES (**4a-j)**

Solvent	Base	Temp. (°C)	Yield (%)
DMF	K_2CO_3	RT	27
ACN	K_2CO_3	RT	21
Acetone	K_2CO_3	RT	–
DMF	NaI + K_2CO_3	0	31
DMF	$(CH_3)_3COK$	RT	89
DMF	$(CH_3)_3COK$	80	34
DMF	CS_2CO_3	RT	69
DMF	CS_2CO_3	100	43
THF	CS_2CO_3	RT	–
ACN	CS_2CO_3	RT	51

N′-(4-methoxybenzylidene)benzohydrazide (1 equiv.), epichlorohydrin (4 equiv.) in the presence of potassium *tert*-butoxide (1.5 equiv.) in DMF for 2 h. To achieve final product, these obtained scaffolds further reacted with diethylamine. It is highly regioselective nucleophilic ring-opening of epoxide in presence of NaOH (catalytic amount) using ethyl acetate as a polar aprotic solvent in heating for 2 h.

Biological activity: The antimicrobial activity data shown in the Tables 2 and 3 display that zone of inhibition values lies in the range of 35 to 0 mm in both solvents. All the synthesized compounds are less active against *S. aureus* and *B. subtilis* than the standard drug ciprofloxacin and more effective on *M. leutus* in DMSO but in DMF, several compounds showed excellent inhibition then standard drug. Compound **5a** (4-methoxy) exhibited significant zone of inhibition even at lower concentration (15 mm, 250 µg/mL) comparatively against *S. aureus* but in DMSO. However, in DMF it did not show any activity. Compounds **5f**, **5h, 5i** and **5j** containing 4-chloro, 3-chloro, thiophene and pyridine as substitution groups, respectively are the prime inhibitors than standard drug for *S. aureus* in DMF. Compound **5b** (4-H), **5h** (3-chloro) and **5i** (thiophene) and **5j** (pyridine) are highly potent against *M. luteus*, even more than the standard drug in DMF. In case of Gram-negative strains, all compounds exhibited poor zone of inhibition in DMSO compare to DMF. Compounds **5i** and **5j** with thiophene and pyridine functional groups respectively is more effective against *E. coli* than others except compound **5g** with 4-fluoro group, which also displayed a great activity at higher concentration. *A. niger* is the most susceptible fungal strain in DMSO but in DMF, all compounds showed significant zone of inhibition. Almost all the compounds exhibited good inhibition compare to Ketoconazole the against the fungal strains in DMF. The compounds **5f** and **5j** were more effective at even minimum concentration (20 mm, 250 µg/mL) and compounds **5f** and **5j** showed highest inhibition at 1000 µg/mL is 25 mm against *S. cerevisie* and **5e** (25 mm, 1000 µg/mL) showed for *A. niger.*

Ketoconazole had recorded to be efficacious against all fungal starins in both solvents. However, the synthesized

TABLE-2
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED BENZOHYDRAZIDE DERIVATIVES (**5a-j**) IN DMSO

Sample code	Zone of inhibition in mm (DMSO)																	
	Antibacterial activity																	
	Gram-positive									Gram-negative								
	S. aureus			*B. subtilis*			*M. leutus*			*E. coli*			*P. aerogenosa*			*S. typhi*		
	250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
5a	15	20	21	15	18	20	18	18	20	15	17	20	–	–	–	13	15	18
5b	–	–	–	15	19	20	–	–	–	–	–	–	–	–	–	–	–	–
5c	–	–	–	13	15	16	–	15	20	–	–	–	–	–	–	11	12	17
5d	10	11	14	10	13	15	–	11	13	–	14	17	–	–	–	–	–	–
5e	12	15	17	11	13	15	–	12	15	–	–	–	–	–	–	–	–	–
5f	7	9	17	10	13	15	13	13	19	13	15	19	–	–	–	–	–	–
5g	10	11	12	–	–	–	–	–	10	12	15	17	–	–	–	–	–	–
5h	–	–	7	10	12	17	12	12	15	10	10	17	–	10	12	13	15	17
5i	–	–	–	–	–	–	–	17	19	–	–	–	–	–	–	15	23	25
5j	–	–	–	10	12	13	–	–	–	–	–	–	10	13	15	–	–	–
Ciprofloxacin	32	32	33	35	36	38	–	2	8	30	32	33	33	34	35	36	31	33

	Antifungal activity (MIC)								
	S. cerevisie			*A. niger*			*Penicillium* sp.		
	250	500	1000	250	500	1000	250	500	1000
5a	15	18	20	–	–	–	10	10	15
5b	–	–	–	–	–	15	–	–	10
5c	11	12	17	–	–	–	–	–	–
5d	15	17	20	–	–	15	–	13	15
5e	–	13	18	–	–	–	–	–	–
5f	–	–	–	10	10	11	–	10	12
5g	–	–	10	–	–	–	–	–	–
5h	–	–	–	–	–	–	–	–	–
5i	–	–	10	–	–	–	–	–	–
5j	8	10	15	–	–	–	–	–	–
Ketoconazole	5	17	23	7	23	33	8	14	28

"–" means there is no zone of inhibition

TABLE-3
ANTIMICROBIAL ACTIVITY OF SYNTHESIZED BENZOHYDRAZIDE DERIVATIVES (**5a-j**) IN DMF

Sample code	Zone of inhibition in mm (DMF)																	
	Antibacterial activity																	
	Gram-positive									Gram-negative								
	S. aureus			*B. subtilis*			*M. leutus*			*E. coli*			*P. aerogenosa*			*S. typhi*		
	250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000	250	500	1000
5a	–	–	–	–	–	–	18	20	20	–	–	–	–	–	17	–	–	–
5b	–	23	26	17	19	21	20	23	25	–	–	–	–	–	–	–	13	15
5c	17	18	20	13	15	17	15	17	19	15	20	24	–	–	–	–	–	–
5d	14	20	25	10	18	18	13	15	17	–	14	17	–	–	–	13	14	18
5e	–	17	20	11	13	17	12	12	13	–	–	–	–	–	–	–	–	–
5f	25	30	31	12	15	19	14	16	20	10	17	19	20	22	25	–	–	12
5g	26	27	30	–	–	–	9	15	19	20	23	29	12	18	22	16	17	19
5h	30	31	35	10	12	15	12	16	20	10	13	15	12	12	15	20	20	22
5i	30	33	35	–	10	12	17	19	20	20	23	27	–	–	–	13	15	18
5j	30	30	31	10	12	15	10	17	20	17	20	27	20	22	25	–	–	–
Ciprofloxacin	32	30	33	35	36	39	–	9	10	30	32	35	32	35	36	28	30	33

	Antifungal activity (MIC)								
	S. cerevisie			*A. niger*			*Penicillium* sp.		
	250	500	1000	250	500	1000	250	500	1000
5a	11	13	15	15	21	23	11	12	15
5b	21	21	23	–	10	15	–	17	21
5c	13	17	22	–	–	–	–	11	17
5d	16	17	20	–	15	20	10	13	16
5e	15	17	19	15	23	25	–	–	–
5f	20	23	25	10	15	17	18	20	22
5g	–	–	–	15	18	19	–	11	19
5h	–	–	–	10	12	15	15	17	20
5i	15	18	20	12	20	20	13	15	20
5j	20	22	25	13	15	15	12	17	20
Ketoconazole	7	18	26	9	26	38	9	17	35

"–" means there is no zone of inhibition

benzohydrazide compounds were active against *A. niger*. Comparison of inhibition of compounds between both the solvents shows that *M. leutus* is the most affected bacteria in both the solvents. DMF exhibited higher inhibition than DMSO. All the results displayed that the zone of inhibition values more than standard drug was considered as promising antifungal agents.

Conclusion

In this study, some novel benzohydrazide compounds were synthesized by regioselective nucleophilic ring-opening reaction by amine using conventional methods at room temperature in the presence of sodium hydroxide as a catalyst in polar aprotic solvent such as ethyl acetate. The synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, elemental analysis and mass spectrometry techniques. This synthetic method proceeds easily with other various heterocyclic benzaldehydes too. The chief interests of this protocol are regioselectivity, short time, excellent yield, simple isolation of product and used chemicals that are radially available in the laboratory. Substituted *N*′-benzylidene-4-(3-(diethylamino)-2-hydroxypropoxy)benzohydrazide derivatives showed excellent antibacterial activity. They are highly potent against chosen fungal strains compared to standard drug in DMF. These compounds can be explored further as potent molecules in the growth of the newer antimicrobial drugs and may play a essential role in the development of other chemotherapeutic agents.

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