Med Chem Res (2012) 21:1237–1244
DOI 10.1007/s00044-011-9634-0

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis, characterization and antimicrobial evaluation of novel derivatives of isoniazid

Manav Malhotra · Sagun Sharma · Aakash Deep

Received: 16 September 2010 / Accepted: 24 March 2011 / Published online: 10 April 2011

Abstract In the present investigation, a series of new Mannich bases were prepared by the reaction of 2-ethoxybenzaldehyde with isoniazid to form acid hydrazone (**3a**). Further, C-Mannich bases of the above acid hydrazone were prepared by aminomethylation with formaldehyde and substituted secondary amines (**3b–3k**). The structures of newly synthesized compounds were evaluated by elemental analyses and spectral (IR, ^{1}H NMR, ^{13}C NMR) studies. All the synthesized compounds were evaluated for their antimicrobial activity. Amoxicillin was used as a standard drug for antibacterial activity while Nystatin was used as a standard drug for antifungal activity. Preliminary pharmacological evaluation revealed that the compound (**3f**, **3i**, **3j**, **3k**) showed better performance against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Candida gabrata.* The result demonstrates the potential and importance of developing new mannich bases which would be effective against resistant bacterial and fungal strain.

Keywords Mannich bases · Hydrazones · Isoniazid · Antibacterial & antifungal activity

M. Malhotra · S. Sharma
Research Scholar, Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Ferozpur Road, Moga 142001, Punjab, India

A. Deep (✉)
Department of Pharmaceutical Sciences, G.V.M. College of Pharmacy, Sonepat 131001, India
e-mail: aakashdeep82@gmail.com

Introduction

Morbidity and mortality caused due to bacterial and fungal infections are the major cause of health problem in developing countries (Qadri *et al.*, 2005; Devasia *et al.*, 2006). Millions of people were infected and around 20,000 deaths were reported in the tropical regions every year because of bacterial infections (Datta *et al.*, 1974). So, there is an urgent need for identification of novel lead structure for designing of new, potent and less toxic agents which ideally shorten the duration of therapy and are effective against the resistant strain (Murphy *et al.*, 2007). Hydrazones belong to the Schiff base family containing azomethine –NHN=CH– protons and they are considered as the important class of compounds for the development of new drugs (Rollas and Kucukguzel, 2007). Hydrazones have been reported to posses antimicrobial (Rollas *et al.*, 2002), antitubercular (Imramovsky *et al.*, 2007; Janin, 2007), antileprotic (Buuhoi *et al.*, 1956), anticonvulsant (Dimmock *et al.*, 2000), analgesic (Lima *et al.*, 2000), anti-inflammatory (Salgin-Goksen *et al.*, 2007; Kalsi *et al.*, 1990), antiplatelet (Silva *et al.*, 2004), anticancer (Savini *et al.*, 2004; Bijev, 2006), antifungal (Lonce *et al.*, 2004), antiviral (Abdel-Aal *et al.*, 2006), antitumor (El-Hawash *et al.*, 2006; Cocco *et al.*, 2006), antibacterial (Capilla *et al.*, 2003), and antimalarial (Walcourt *et al.*, 2004) activities. The first hydrazine derivative is characterized by Fischer and Deutsch (1875). Hydrazones have been received much attention and many studies have been reported due to their chemotherapeutic value in the development of novel antimicrobial agents (Papakonstantinou *et al.*, 2002; Vacini *et al.*, 2002; Masunari and Tavares, 2007; Joshi *et al.*, 2008; Kumar *et al.*, 2009; Ozdemir *et al.*, 2009). Hydrazones are used as plasticizers, polymerization initiators, antioxidants, and in the determination

of carbonyl containing compound (Singh *et al*., 1982). Antimicrobial resistance to a drug can be overcome by designing the new derivatives (Wechter *et al*., 1975). Further, pharmacokinetic and cellular permeability of the drug can be increased by derivatization to bioreversible form of this drug, namely hydrazones (Maccari *et al*., 2002). The Mannich base of isonicotinoyl hydrazone has improved lipid solubility (Joshi *et al*., 2004). Mannich reaction is a three-component condensation reaction involving active hydrazone containing compound, formaldehyde, and a secondary amine (Sujith *et al*., 2009). It is believed that the Mannich base functional group increases the lipophilicity of parent amines and amides and results into enhancement of absorption through biomembranes (Gamal El-Din *et al*., 2009). The lipophilicity of mannich bases enables them to cross bacterial and fungal membranes. So, looking at the antimicrobial importance of hydrazone and its mannich bases, prompted us to synthesize some new derivatives of Mannich bases. So, considering this entire concept, we report the synthesis, characterization, and antimicrobial study of hydrazone and its mannich bases.

Chemistry

The synthesis of target compounds were carried outline in synthetic Scheme 1. Compounds **3a–3k** wwere readily prepared in good yields and purity. Equimolar quantity of 2-ethoxybenzaldehyde (1.50 g, 0.01 mol) and Isoniazid (1.37 g, 0.01 mol) in 15 ml of absolute ethanol was refluxed for 7 h to form 2-ethoxybenzylidene isonicotinohydrazide. The completion of reaction was confirmed by thin layer chromatography (TLC). Then 2-ethoxybenzylidene isonicotinohydrazide (646 mg, 0.0024 mol) along with (0.1 ml, 0.0036 mol) of formaldehyde and (0.0024 mol) of substituted secondary amines were refluxed in the presence of 50 ml of super dry ethanol and the pH was adjusted to four with hydrochloric acid. The purity of the compounds was checked by TLC, elemental analyses and characterized by spectral data. In general, IR spectra of all compounds **3a–3k** showed absorption band at around 3284–3257, 2869–2838, 2929–2926, 1676–1665, 1669–1651, 1582–1539, 1188–1118, 1088–1049 cm^{-1} regions, conforming the presence of NH, CH_2, CH, C=N, C=O, C=C, C–N, and C–O, respectively. The ^{1}H NMR spectra, the signals of the respective prepared derivatives were confirmed on the basis of their chemical shifts, multiplicities, and coupling constants. The spectra of most compounds showed the characteristic NH proton δ 12.05–11.74 ppm, 1H proton of –N=C–H at δ 8.54–8.31 ppm, 4H proton of pyridine were at around δ 8.89–7.32 ppm, and characteristic protons of benzylidene at δ 7.82–6.94 ppm. ^{13}C-NMR spectra of compounds **3a–3k** characteristic C=O signals appeared at around δ 163.59–163.18 ppm, pyridine δ 149.81–122.12 ppm, –N=C–H δ 143.66–143.13 ppm, benzylidene δ 158.13–114.75 ppm, δ O–CH_2 65.57–64.15 ppm, δ Ar–CH_2–N 55.91–45.13 ppm.

Materials and methods

Melting points of the synthesized compounds were determined in open-glass capillaries on Stuart SMP10 melting point apparatus and were uncorrected. The purity of the compounds was checked by thin layer chromatography (TLC). Silica gel plates kiesel gel 0.25 mm, 60G F254, precoated sheets obtained from Merck, Darmstadt (Germany) were used for TLC and the spots were visualized by iodine vapors/ultraviolet light as visualizing agent. The IR spectra (υ, cm^{-1}) were obtained with a Perkin-Elmer 1600 FTIR spectrometer in KBr pellets. ^{1}H-NMR spectra (δ, ppm) were recorded in DMSO-d_6 solutions on a Varian-Mercury 300 MHz spectrometer using tetramethylsilane as the internal reference. ^{13}C-NMR spectra were recorded on in DMSO-d_6 solutions on a Bruker Avance II 400 spectrometer at 400 MHz using tetramethylsilane as the internal reference. Elemental analyses were performed on an ECS 4010 Elemental Combustion System. The necessary chemicals were purchased from Loba Chemie.

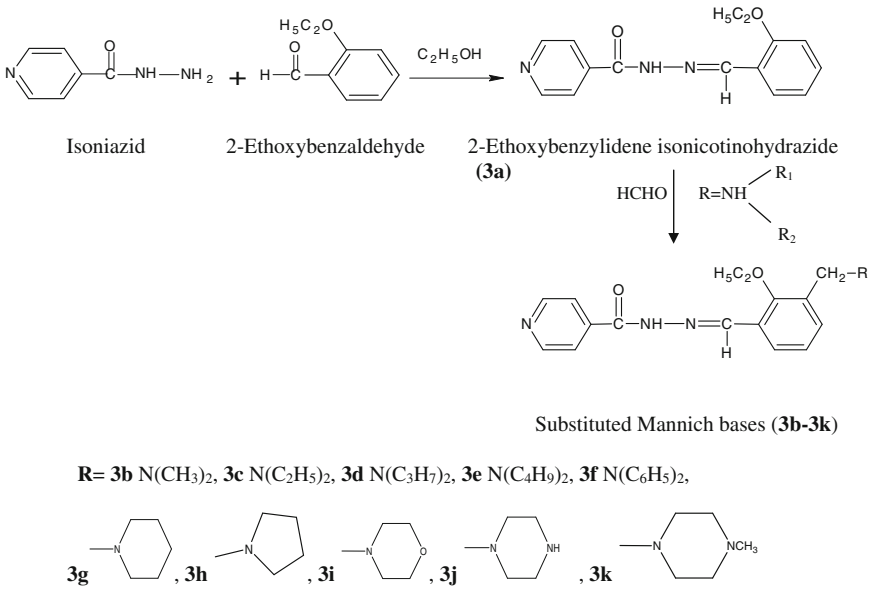
Synthesis of 2-ethoxybenzylidene Isonicotinohydrazide

A mixture of 2-ethoxybenzaldehyde (1.50 g, 0.01 mol) and isoniazid (1.37 g, 0.01 mol) in 15 ml of super dry ethanol(method of preparation of dry ethanol: Take 1 l of ethanol and add 25 g of magnesium metals. Reflux until the metal is consumed (add a few drops of chloroform if it doesn't start to get cloudy).It will take a good 24 h to convert the metal to magnesium ethoxide. Then just distill the ethanol off. It will be very dry) was refluxed for 7 h. The completion of reaction was confirmed by TLC. The reaction mixture was then poured onto ice cold water and the precipitate obtained was filtered and dried in oven at low temperature. The product was recrystallised from absolute ethanol.

*N-(2-ethoxybenzylidine)Isonicotinohydrazide (**3a**)*

Yield 68%; m.p. 192–195°C; IR (KBr; cm^{-1}): 3261, 2934, 2865, 2838, 1674, 1652, 1561, 1116, 1064. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 12.05 (s, 1H, –NH–N=), 8.82 (d, 2H, pyridine, $J = 4.5$ Hz), 8.74 (s, 1H, –N = C–H), 7.88 (d, 2H, pyridine, $J = 4.2$ Hz), 7.82 (d, 2H, benzylidene, $J = 7.8$ Hz), 7.40 (d, 2H, benzylidene, $J = 7.5$), 3.86 (m, 2H, OCH_2), 1.28 (t, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.45, 157.32, 149.81,

Scheme 1 Synthetic pathway for the formation of the title compounds



143.17, 139.87, 131.73, 129.88, 122.69, 120.51, 117.33, 114.75, 65.32, 14.92. Anal.: Calcd. for $C_{15}H_{15}N_3O_2$ (269.30): C 66.90, H 5.61, N 15.60. Found: C 65.95, H 5.63, N 15.53.

Synthesis of substituted mannich bases (**3b**–**3k**)

The 2-ethoxy-benzylidene isonicotinohydrazide (646 mg, 0.0024 mol) along with (0.1 ml, 0.0036 mol) of formaldehyde and (0.0024 mol) of substituted secondary amines was placed in 100 ml round bottom flask to which 50 ml of super dry ethanol was added and the pH was adjusted to four with hydrochloric acid and refluxed for 38–43 h. The completion of reaction was confirmed by TLC. The reaction mixture was then concentrated on water bath and allowed it to cool at room temp for half an hour to which diethyl ether was added. The reaction mixture was kept for 3–5 h in refrigerator and filtered and washed with *n*-hexane. The products were recrystallised from absolute ethanol.

*N-3-((dimethyamino)methyl)-2-ethoxybenzylidene)isonicotinohydrazide (**3b**)*

Yield 52%; m.p. 225–227°C; IR (KBr; cm^{-1}): 3267, 2924, 2869, 2845, 1676, 1654, 1562, 1118, 1058. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.82 (s, 1H, –NH–N=), 8.69 (d, 2H, pyridine, $J = 4.2$ Hz), 8.44 (s, 1H, –N=C–H), 7.74 (d, 2H, pyridine, $J = 3.8$ Hz), 7.58 (d, 2H, benzylidene, $J = 3.2$ Hz), 7.28 (t, 1H, benzylidene), 3.82 (m, 2H, OCH_2), 3.57 (s, 2H, Ar–CH_2–N), 3.32 (s, 6H, N–$2CH_3$), 1.12 (t, 3H, OCH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.59, 158.13, 149.37, 143.41, 139.79, 132.74, 127.39, 122.38, 121.52, 119.87, 115.18, 64.57, 54.91, 44.74, 13.87. Anal.: Calcd. for $C_{18}H_{24}N_4O_2$. (326.39) C 66.24, H 6.79, N 17.17. Found: C 66.18, H 6.75, N 17.27.

*N-3-((diethyamino)methyl)-2-ethoxybenzylidene)isonicotinohydrazide (**3c**)*

Yield 55%; m.p. 220–222°C; IR (KBr; cm^{-1}): 3284, 2958, 2863, 2842, 1671, 1658, 1555, 1124, 1067. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.74 (s, 1H, –NH–N=), 8.71 (d, 2H, pyridine, $J = 4.2$ Hz), 8.39 (s, 1H, –N=C–H), 7.69 (d, 2H, pyridine, $J = 3.9$ Hz), 7.64 (d, 2H, benzylidene, $J = 3.5$ Hz), 7.12 (t, 1H, benzylidene), 3.91 (m, 2H, OCH_2), 3.62 (s, 2H, Ar–CH_2–N), 2.87 (m, 4H, N–$2CH_2$), 1.14 (m, 9H, $3CH_3$); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.34, 157.64, 149.39, 143.39, 139.74, 131.98, 127.71, 122.82, 121.69, 119.74, 115.19, 64.84, 52.17, 48.63, 14.81, 13.39. Anal.: Calcd. for $C_{20}H_{26}N_4O_2$. (354.45) C 67.77, H 7.39, N 15.81. Found: C 67.82, H 7.38, N 15.77.

*N-3-((dipropylamino)methyl)-2-ethoxybenzylidene)isonicotinohydrazide (**3d**)*

Yield 55%; m.p. 215–217°C; IR (KBr; cm^{-1}): 3265, 2954, 2853, 2841, 1671, 1658, 1541, 1118, 1052. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.86 (s, 1H, –NH–N=), 8.64 (d, 2H, pyridine, $J = 4.2$ Hz), 8.39 (s,1H,–N=C–H), 7.93 (d, 2H, pyridine, $J = 3.9$ Hz), 7.34 (d, 2H, benzylidene, $J = 3.4$ Hz), 7.11 (t, 1H, benzylidene), 3.78 (m, 2H, OCH_2), 3.69 (s, 2H, Ar–CH_2–N), 2.18 (m, 8H, N–$4CH_2$), 1.12 (m, 9H, $3CH_3$); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.18, 157.59, 149.18, 143.23, 139.93, 131.81, 128.12, 122.57, 121.59, 120.27, 116.61, 64.86, 56.18, 51.17, 22.54, 14.88, 11.52. Anal.: Calcd. for $C_{22}H_{30}N_4O_2$. (382.50) C 69.08, H 7.91, N 14.65. Found: C 69.15, H 7.85, N 14.64.

*N-3-((dibutylamino)methyl-2-ethoxybenzylidene)isonicotinohydrazide (**3e**)*

Yield 52%; m.p. 208–210°C; IR (KBr; cm^{-1}): 3266, 2958, 2851, 2843, 1674, 1655, 1539, 1124, 1062. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.75 (s, 1H, –NH–N=), 8.69 (d, 2H, pyridine, $J = 4.1$ Hz), 8.39 (s, 1H, –N=C–H), 7.89 (d, 2H, pyridine, $J = 3.8$ Hz), 7.58 (d, 2H, benzylidene, $J = 3.2$ Hz), 7.34 (t, 1H, benzylidene), 3.72 (m, 2H, OCH_2), 3.66 (s, 2H, Ar–CH_2–N), 2.26 (m, 4H, N–2CH_2), 1.75 (m, 8H, 4CH_2), 1.14 (t, 9H, 3CH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.27, 157.19, 149.37, 143.13, 139.86, 131.77, 128.57, 122.12, 120.18, 116.88, 64.91, 55.26, 51.28, 32.54, 21.19, 14.91, 13.55. Anal.: Calcd. for $C_{24}H_{34}N_4O_2$. (410.55) C 70.21, H 8.35, N 13.65. Found: C 70.28, H 8.38, N 13.55.

*N-(3((diphenylamino)-methyl)-2-ethoxybenzylidene)isonicotinohydrazide (**3f**)*

Yield 53%; m.p. 199–201°C; IR (KBr; cm^{-1}): 3269, 2989, 2865, 2841, 1669, 1655, 1558, 1139, 1075. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.92 (s, 1H, –NH–N=), 8.74 (d, 2H, pyridine, $J = 4.5$ Hz), 8.56 (s, 1H, –N=C–H), 7.39 (d, 2H, pyridine, $J = 4.2$ Hz), 7.24–6.94 (m, 13 Ar–H, benzylidene), 3.98 (m, 2H, OCH_2), 3.62 (s, 2H, Ar–CH_2–N), 1.27 (s, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.19, 156.27, 149.83, 143.34, 139.87, 129.72, 127.81, 122.59, 119.45, 118.64, 116.77, 65.15, 45.13, 15.24. Anal.: Calcd. for $C_{28}H_{26}N_4O_2$. (450.21) C 74.65, H 5.82, N 12.44. Found: C 74.59, H 5.88, N 12.44.

*N-(2-ethoxy-3-((piperidine-1-yl)methyl)benzylidene)isonicotinohydrazide (**3g**)*

Yield 58%; m.p. 191–193°C; IR (KBr; cm^{-1}): 3265, 2963, 2864, 2842, 1674, 1649, 1561, 1131, 1055. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.92 (s, 1H, –NH–N=), 8.74 (d, 2H, pyridine, $J = 4.2$ Hz), 8.56 (s, 1H, –N=C–H), 7.94 (d, 2H, pyridine, $J = 3.8$ Hz), 7.28 (d, 2H, benzylidene, $J = 3.2$ Hz), 7.13 (t, 1H, benzylidene), 3.84 (m, 2H, OCH_2), 3.47 (s, 2H, Ar–CH_2–N), 2.65 (t, 4H, N–2CH_2, piperidine), 1.24 (m, 6H, 3CH_2, piperidine), 1.15 (t, 3H, 3CH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.34, 157.18, 149.38, 143.27, 139.84, 131.22, 128.37, 122.74, 121.18, 120.74, 116.93, 64.94, 55.16, 51.77, 25.17, 14.81. Anal.: Calcd. for $C_{21}H_{26}N_4O_2$. (366.46) C 68.83, H 7.15, N 15.29. Found: C 68.58, H 7.18, N 15.24.

*N-(2-ethoxy-3-((pyrrolidin-1-yl)methyl)benzylidene)isonicotinohydrazide (**3h**)*

Yield 57%; m.p. 196–198°C; IR (KBr; cm^{-1}): 3257, 2959, 2862, 2842, 1669, 1655, 1561, 1187, 1088. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.88 (s, 1H, –NH–N=), 8.79 (d, 2H, pyridine, $J = 4.5$ Hz), 8.42 (s, 1H, –N=C–H), 7.78 (d, 2H, pyridine, $J = 4.3$ Hz), 7.59 (d, 2H, benzylidene, $J = 3.2$ Hz), 7.19 (t, 1H, benzylidene), 3.75 (m, 2H, OCH_2), 3.55 (s, 2H, Ar–CH_2–N), 2.37 (d, 4H, N–2CH_2, pyrrolidine), 1.37 (m, 4H, 2CH_2, pyrrolidine), 1.25 (t, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.44, 157.28, 149.53, 143.18, 139.47, 131.39, 128.74, 122.91, 121.67, 120.19, 116.76, 64.79, 56.89, 51.27, 26.73, 14.82. Anal.: Calcd. for $C_{20}H_{24}N_4O_2$. (352.19) C 68.16, H 6.86, N 15.90. Found: C 68.15, H 6.85, N 15.92.

*N-(2-ethoxy-3-((morpholinomethyl)benzylidene) isonicotinohydrazide (**3i**)*

Yield 45%; m.p. 219–221°C; IR (KBr; cm^{-1}): 3264, 2989, 2863, 2845, 1668, 1655, 1582, 1184, 1079. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.88 (s, 1H, –NH–N=), 8.89 (d, 2H, pyridine, $J = 4.3$ Hz), 8.46 (s, 1H, –N=C–H), 7.79 (d, 2H, pyridine, $J = 3.8$ Hz), 7.38 (d, 2H, benzylidene, $J = 3.1$ Hz), 7.19 (t, 1H, benzylidene), 3.92 (m, 2H, OCH_2), 3.67 (s, 2H, Ar–CH_2–N), 3.57 (t, 4H, O–2CH_2, morpholine), 2.42 (t, 4H, 2CH_2, morpholine), 1.25 (t, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.19, 157.34, 149.53, 143.66, 139.48, 131.67, 128.53, 122.53, 121.91, 120.49, 116.13, 67.51, 54.19, 51.29, 15.22. Anal.: Calcd. for $C_{20}H_{24}N_4O_3$. (368.43) C 65.20, H 6.57, N 15.21. Found: C 65.23, H 6.47, N 15.28.

*N-(2-ethoxy-3-((piperazin-1-yl)methyl)benzylidene)isonicotinohydrazide (**3j**)*

Yield 49%; m.p. 205–207°C; IR (KBr; cm^{-1}): 3259, 2984, 2865, 2841, 1673, 1658, 1567, 1188, 1049. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.81 (s, 1H, –NH–N=), 8.69 (d, 2H, pyridine, $J = 4.1$ Hz), 8.69 (s, 1H, –N=C–H), 7.59 (d, 2H, pyridine, $J = 3.7$ Hz), 7.38 (d, 2H, benzylidene, $J = 3.2$ Hz), 7.19 (t, 1H, benzylidene), 3.78 (m, 2H, OCH_2), 3.65 (s, 2H, Ar–CH_2–N), 2.62 (m, 8H, N–4CH_2, piperazine), 2.37 (m, 1H, NH, piperazine), 1.28 (t, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.25, 157.55, 149.67, 143.18, 139.67, 131.54, 128.18, 122.86, 121.51, 120.55, 116.77, 64.59, 55.86, 51.67, 46.35, 15.23. Anal.: Calcd. for $C_{20}H_{25}N_5O_2$. (367.44) C 65.37, H 6.86, N 19.06. Found: C 65.56, H 6.72, N 19.01.

*N-(2-ethoxy-3-((4-methylpiperazin-1-yl)methyl)benzylidene)isonicotinohydrazide (**3k**)*

Yield 51%; m.p. 210–212°C; IR (KBr; cm^{-1}): 3264, 2977, 2862, 2842, 1665, 1651, 1555, 1158, 1078. ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm): 11.95 (s, 1H, –NH–N=), 8.77 (d, 2H, pyridine, $J = 4.2$ Hz), 8.39 (s,1H, –N=C–H),

7.69 (d, 2H, pyridine, J = 3.9 Hz), 7.34 (d, 2H, benzylidene, $J = 3.3$ Hz), 7.13 (t, 1H, benzylidene), 3.89 (m, 2H, OCH_2), 3.66 (s, 2H, Ar–CH_2–N), 2.47 (t, 8H, N–4CH_2, piperazine), 2.12 (s, 3H, NCH_3, piperazine), 1.22 (t, 3H, CH_3); ^{13}C-NMR (400 MHz, DMSO d_6, δ ppm): 163.28, 157.51, 149.34, 143.27, 139.44, 131.84, 128.53, 122.92, 121.47, 120.23, 116.75, 64.86, 56.13, 52.17, 51.25, 43.71, 15.18. Anal.: Calcd. for $C_{21}H_{27}N_5O_2$. (381.47) C 66.12, H 7.13, N 18.36. Found: C 66.25, H 7.18, N 18.18.

Antimicrobial activity

Culture media

Two specific media were used for detecting the antimicrobial activity, nutrient agar medium was used for bacterial growth [beef extract, 3 g; bacteriological peptones, 5 g; agar, 20 g, the pH was adjusted to 6.2 ± 0.2 at 25 (±2)°C], while malt extract agar (MEA) for fungal isolates [malt extract, 20 g; bacteriological peptone, 5 g; agar, 20 g, the pH was adjusted to 5.4 ± 0.2 at 25 (±2)°C]. Each medium was prepared by dissolving the solid ingredient in 1 l of cold distilled water and then heated to 60–70°C with stirring. Media were sterilized by autoclaving at 121°C (1.5 atm) for 15–20 min (Atlas 1993).

Antimicrobial assays

By diffusion agar technique, the antibacterial and antifungal potentialities against several species are expressed as the measurement of diameter of their inhibition zone. Hot-plate diffusion method was used; six equidistant (1 cm diameter) holes were made using sterile cork borer in malt extract agar and nutrient agar sterile plates (10 × 10 cm), which had previously been seeded with tested bacterial and fungal isolates. Holes are filled with 100 μg/ml concentration of each of the synthesized compounds after completely dissolving in DMSO. Controlled holes were filled with DMSO solvent. Plates were left in a cooled incubator at 37 (±2)°C for bacterial isolates and incubation at 28 (± 2)°C for fungal isolates used. Inhibition zones developed due to active ingredients were measured after 24–48 h of incubation time. *Amoxicillin* was used as standard antibacterial agent while *Nystatin* was used as a standard antifungal agent.

Minimum inhibitory concentration (MIC) was determined by broth dilution technique. The Nutrient Broth contained logarithmic serially two fold diluted amount of test compound and controls. The inoculum size was approximately 10^6 colony forming units (CFU/ml). The tubes were incubated at 37 ± 1°C for 24 h (bacteria) and 25°C for 7 days (*A. Niger*) and the growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of microbes was regarded as minimum inhibitory concentrations (MIC). To obtain the minimum fungal concentration (MFC), 0.1 ml volume was taken from each tube and spread on agar plates. The number of cfu was counted after 3 days of incubation at 30°C. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. MIC_{50} were calculated mathematically depending on the number of inhibited colonies of the

Table 1 Zone of inhibition of the tested compounds

Compound	Concentration (μg/ml)	Zone of inhibition (mm)					
		Gram positive bacteria		Gram negative bacteria		Fungal strain	
		B. subtilis (ATCC 25923)	*S. aureus* (ATCC 29213)	*P. aeruginosa* (ATCC 27863)	*E. coli* (ATCC 25922)	*C. albicans* (ATCC 10231)	*C. gabrata* (ATCC 10233
3a	100	15	19	14	18	13	11
3b	100	17	21	15	20	13	14
3c	100	19	21	16	20	15	14
3d	100	21	22	18	22	15	15
3e	100	21	23	19	24	15	15
3f	100	26	31	22	32	21	22
3g	100	23	25	20	25	16	16
3h	100	22	26	20	27	18	19
3i	100	27	32	22	31	21	21
3j	100	27	34	23	33	24	23
3k	100	26	33	22	32	22	20
Amoxicillin	50	25	30	21	30	–	–
Nystatin	50	–	–	–	–	–	–

medium with the respective Mannich base compounds or standard drug dilution compared to the control medium colonies without drugs.

Result and discussion

The antimicrobial sensitivity testing of the synthesized compounds assayed using cup plate technique in the nutrient agar at 100 μg/ml concentration was shown in Table 1. Amoxicillin standard was active at 50 μg/ml on all the Gram (+ve) and Gram (−ve) bacteria. From the antibacterial screening, it was concluded that compounds **3f, 3i, 3j,** and **3k** show larger zone of inhibition as compared to standard drug amoxicillin against *Bacillus subtilis* (26, 27, 27, 26 mm), *Staphylococcus aureus* (31, 32, 34, 33 mm), and Gram (−ve) bacteria *Pseudomonas aeruginosa* (22, 22, 23, 22 mm) and *Escherichia coli* (32, 31, 33, 32 mm).

Table 2 MIC_{50} of tested compounds

Compound	MIC_{50}					
	Gram positive bacteria		Gram negative bacteria		Fungal strain	
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*	*C. gabrata*
3a	25.6	12.8	12.8	25.6	25.6	25.6
3b	12.8	12.8	12.8	12.8	12.8	25.6
3c	12.8	12.8	12.8	12.8	12.8	25.6
3d	12.8	6.4	6.4	6.4	12.8	12.8
3e	12.8	6.4	6.4	6.4	12.8	12.8
3f	6.4	3.2	6.4	3.2	6.4	12.8
3g	12.8	6.4	6.4	6.4	12.8	12.8
3h	12.8	6.4	6.4	3.2	6.4	12.8
3i	6.4	3.2	6.4	3.2	6.4	12.8
3j	6.4	3.2	6.4	3.2	6.4	12.8
3k	6.4	3.2	6.4	3.2	6.4	12.8
Amoxicillin	3.2	1.62	3.2	1.6	–	–
Nystatin	–	–	–	–	3.2	6.4

Table 3 MIC and MFC of tested compounds

Compound	MIC and MFC							
	Gram positive bacteria		Gram negative bacteria		Fungal strain			
	B. subtilis	*S. aureus*	*P. aeruginosa*	*E. coli*	*C. albicans*		*C. gabrata*	
						MFC		MFC
3a	50	25.0	25.0	50.0	50.0	50	25.0	100
3b	50	25.0	25.0	25.0	25.0	100	25.0	100
3c	25.0	25.0	25.0	25.0	25.0	25.0	25.0	100
3d	25.0	25.0	12.5	12.5	25.0	50	12.5	100
3e	25.0	12.5	12.5	12.5	25.0	100	12.5	100
3f	12.5	6.25	12.5	6.25	12.5	25	12.5	50
3g	25.0	12.5	12.5	12.5	12.5	100	12.5	100
3h	25.0	12.5	12.5	6.25	12.5	25	12.5	100
3i	12.5	6.25	12.5	6.25	12.5	25	12.5	50
3j	12.5	3.12	12.5	6.25	12.5	25	12.5	50
3k	12.5	6.25	12.5	6.25	12.5	25	12.5	50
Amoxicillin	6.25	3.12	6.25	3.12	–	–	–	–
Nystatin	–	–	–	–	6.25	12.5	12.5	25.0

MIC Minimum inhibitory concentration

MFC Minimum fungicidal concentration

Compounds **3g** and **3h** showed nearly moderate zone of inhibition as compared to amoxicillin,while the rest of compounds have shown a lesser amount of antibacterial activity as compared to the standard drug. Nystatin standard was active at 50 μg/ml on most of the fungal strain. From the antifungal screening, it was concluded that compounds **3f, 3i, 3j,** and **3k** showed larger zone of inhibition as compared to standard drug nystatin against *Candida albicans* (21, 21, 24, 22 mm) and *Candida gabrata* (22, 21, 23, 20 mm). Compounds **3g** and **3h** showed nearly moderate zone of inhibition as compared to nystatin, while the rest of compounds showed a lesser amount of antifungal activity as compared to the standard drug nystatin. MIC_{50} representing the concentration which is able to inhibit 50% growth of microbes was given in Table 2. MFC was defined as the lowest drug concentration at which 99.9% of the inoculums were killed. The minimum inhibitory concentration and minimum fungicidal concentration was given in Table 3.

Conclusion

A series of mannich bases were synthesized for their antimicrobial activity. The highest antibacterial activity against Gram positive species *Bacillus subtilis, Staphylococcus aeuras* and Gram negative species *P. aeruginosa, E. coli* were shown by compounds **3f**, **3i**, **3j**, and **3k**. The compounds **3f, 3i, 3j,** and **3k** also exhibited high antifungal activity against *C. albicans* and *C. gabrata* species. So, it was concluded that antimicrobial activity increases with increase in chain length from dimethyl amine to dibutyl amine. So, the significant antimicrobial activity of compound may be due to the presence of diphenyl amine, morpholine, piperazine, and *N*-methyl piperazine moiety in addition to hydrazide functional group.

References

Abdel-Aal MT, El-sayed WA, El-ashry EH (2006) Synthesis and antiviral evaluation of some sugar arylglycinoylhydrazones and their oxadiazoline derivatives. Arch Pharm Chem Life Sci 339:656–663. doi:10.1002/ardp.200600100

Atlas RM (1993) Handbook of microbiological media, vol 278. CRC Press, Boca Raton, p 785

Bijev A (2006) New heterocyclic hydrazones in the search of antitubercular agents: synthesis and in vitro evaluations. Lett Drug Des Discov 3:506–512

Buuhoi NP, Xuong ND, Tien NB (1956) Compounds of potential interest for the chemotherapy of leprosy. J Org Chem 21:415–418. doi:10.1021/jo01110a010

Capilla J, Serena C, Javier F, Ortoneda T, Guarro J (2003) Efficacy of voriconazole in treatment of systemic scedosporiosis in neutropenic mice. Antimicrob Agents Chemother 47:3976–3978. doi:10.1128/AAC.47.12.3976-3978.2003

Cocco MT, Congiu C, Lilliu V, Onnis V (2006) Synthesis and in vitro antitumoral activity of new hydrazinopyrimidine-5-carbonitrile derivatives. Bioorg Med Chem 14:366–372. doi:10.1016/j.bmc.2005.08.012

Datta DV, Singh SA, Chhutani PN (1974) Treatment of amebic liver abscess with emetine hydrochloride, niridazole, and metronidazole A controlled clinical trial. Am J Trop Med Hyg 23:586–589

Devasia RA, Jones TF, Ward J, Stanfford L, Hardin H, Bopp C, Beatty M, Mintz E, Schaffner W (2006) Endemically acquired foodborne outbreak of enterotoxin-producing *Escherichia coli* serotype O169:H41. Am J Med 119:7–10. doi:10.1016/j.amjmed.2005.07.063

Dimmock JR, Vasishtha SC, Stables JP (2000) Anticonvulsant properties of various acetylhydrazones, oxamoylhydrazones and semicarbazones derived from aromatic and unsaturated carbonyl compounds. Eur J Med Chem 35:241–248. doi:10.1016/S0223-5234(00)00123-9

El-Hawash SAM, Abdel WAE, El-Dewellawy MA (2006) Cyanoacetic acid hydrazones of 3-and 4-acetylpyridine and some derived ring systems as potential antitumor and anti-HCV agents. Arch Pharm Chem Life Sci 339:14–23. doi:10.1002/ardp.200500161

Fischer E, Deutsch Ber (1875) Ueber aromatische hydrazinverbindungen. Bot Ges 8:589–591

Gamal El-Din AR, Hatem AS, Gamal MG (2009) Design, synthesis, antibacterial activity and physicochemical parameters of novel N-4-piperazinyl derivatives of norfloxacin. Bioorg Med Chem 17:3879–3886. doi:10.1016/j.bmc.2009.04.027

Imramovsky A, Polanac S, Vinsova J, Kocevar M, Jampitek J, Reckova Z, Kaustova JA (2007) A new modification of antitubercular active molecules. Bioorg Med Chem 15:2513–2551. doi:10.1016/j.bmc.2007.01.051

Janin Y (2007) Antituberculosis drugs: ten years of research. Bioorg Med Chem 15:2479–2513. doi:10.1016/j.bmc.2007.01.030

Joshi C, Khosla N, Tiwari P (2004) In vitro study of some medicinally important Mannich bases derived from antitubercular agent. Bioorg Med Chem 14:571–576. doi:10.1016/j.bmc.2003.11.001

Joshi SD, Vagdevi HM, Vaidya VP (2008) Synthesis of new 4-pyrrol-1yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring systems: A novel class of potential antibacterial and antitubercular agents. Eur J Med Chem 43:1989–1996. doi:10.1016/j.ejmech.2007.11.016

Kalsi R, Shrimali M, Bhalla TN, Barthwal JP (1990) Synthesis and anti-inflammatory activity of indolyl azetidinones. Ind J Pharm Sci 52:129–134

Kumar P, Narasimhan B, Sharma D, Judge V, Narang R (2009) Hansch analysis of substituted benzoic acid benzylidene/furan-2yl-methylene hydrazides as antimicrobial agent. Eur J Med Chem 44:1853–1863. doi:10.1016/j.ejmech.2008.10.034

Lima PC, Lima Lm, Silva KC, Leda PH, Miranda ALP, Fraga CAM, Barreiro EJ (2000) Synthesis and analgesic activity of novel *N*-acylarylhydrazones and isosters, derived from natural safrole. Eur J Med Chem 35:187–203. doi:10.1016/S0223-5234(00)00120-3

Loncle C, Brunel JM, Vidal N, Herbomez MD, Letourneux Y (2004) Synthesis and antifungal activity of cholesterol-hydrazone derivatives. Eur J Med Chem 39:1067–1071. doi:10.1016/j.ejmech.2004.07.005

Maccari R, Ottana R, Monforte F, Vigorita MG (2002) In vitro antimycobacterial activities of 2′-monosubstituted isonicotinohydrazides and their cyanoborane adducts. Antimicrob Agents Chemother 46:294–299. doi:10.1128/AAC.46.2.294-299.2002

Masunari A, Tavares LC (2007) A new class of nifuroxazide analogues: synthesis of 5-nitrothiophene derivatives with antimicrobial activity against multidrug-resistant *Staphylococcus aureus*. Bioorg Med Chem 15:4229–4236. doi:10.1016/j.bmc.2007.03.068

Murphy ST, Case HL, Ellsworth E, Hagen S, Husband M, Jonnides T, Limberakis C, Marotti KR, Ottolini AM, Rauckhorst M, Starr J, Stier M, Taylor C, Zhu T, Blasser A, Denny WA, Smailic Lu GL, JB Rivault F (2007) The synthesis and biological evaluation of novel series of nitrile-containing fluoroquinolones as antibacterial agents. Bioorg Med Chem Lett 17:2150–2155. doi:10.1016/j.bmcl.2007.01.090

Ozdemir AG, Turan-Zitouni G, Kaplancikli ZA, Tunali Y (2009) Synthesis and biological activities of new hydrazide derivatives. J Enzym Inhib Med Chem 24:825–831. doi:10.1080/14756360802399712

Papakonstantinou S, Pouli N, Marakos P, Chytyroglou-Ladas A (2002) Synthesis antimicrobial and antifungal activity of some new 3-substituted derivatives of 4-(2,4-dichlorophenyl)-5-adamantyl-1H-1,2,4-triazole. Farmaco 57:973–977. doi:10.1016/S0014-827X(02)01227-2

Qadri F, Svennerholm AM, Faruque ASG, Sack RB (2005) Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin Microbiol Rev 18:465–483. doi:10.1128/CMR.18.3.465-483.2005

Rollas S, Kucukguzel SG (2007) Biological activities of hydrazone derivatives. Molecules 12:1910–1939

Rollas S, Gulerman N, Edinz H (2002) Synthesis and antimicrobial activity of some new hydrazones of 4-fluorobenzoic acid hydrazide and 3-acetyl-2, 5-disubstituted-1, 3, 4-oxadiazolines. Farmaco 57:171–174. doi:10.1016/S0014-827X(01)01192-2

Salgin-Goksen U, Gokhan-Kelekci N, Goktas O, Koysal Y, Kılıc E, Isik S, Aktay G, Ozalp ML (2007) Acylthiosemicarbazides, 1,2,4-triazole-5(4H)-thiones, 1,3,4-thiadiazoles and hydrazones containing 5-methyl-2-benzoxazolinones: Synthesis, analgesic-anti-inflammatory and antimicrobial activities. Bioorg Med Chem 15:5738–5751. doi:10.1016/j.bmc.2007.06.006

Savini L, Chiasserini L, Travagli V, Pellerano C, Novellino E, Consentino S, Pisano MB (2004) New α-(*N*)-heterocyclichydrazones: evaluation of anticancer, anti-HIV and antimicrobial activity. Eur J Med Chem 39:113–122. doi:10.1016/j.ejmech.2003.09.012

Silva GA, Costa LMM, Brito FCF, Miranda ALP, Barreiro EJ, Fraga CAM (2004) New class of potent antinociceptive and antiplatelet 10H-phenothiazine-1-acylhydrazone derivatives. Bioorg Med Chem 12:3149–3158. doi:10.1016/j.bmc.2004.04.009

Singh RB, Jain P, Singh RP (1982) Hydrazones as analytical reagents: a review. Talanta 29:77–84. doi:10.1016/0039-9140(82)80024-6

Sujith KV, Jyothi NR, Prashanth S, Balakrishna K (2009) Regioselective reaction: synthesis and pharmacological study of Mannich bases containing ibuprofen moiety. Eur J Med Chem 44:3697–3702. doi:10.1016/j.ejmech.2009.03.044

Vicini P, Zani F, Cozzini P, Doytchinova I (2002) Hydrazones of 1,2-benzisothiazole hydrazides: synthesis, antimicrobial activity and QSAR investigations Hydrazone of 1,2-benzisothiazole hydrazides: synthesis, antimicrobial activity and QSAR investigation. Eur J Med Chem 37:553–564. doi:10.1016/S0223-5234(02)01378-8

Walcourt A, Loyevsky M, Lovejoy DB, Gordeuk VR, Richardson DR (2004) Novel aroylhydrazone and thiosemicarbazone iron chelators with anti-malarial activity against chloroquine resistant and sensitive parasites. Int J Biochem Cell Biol 36:401–407. doi:10.1016/S1357-2725(03)00248-6

Wechter WJ, Johnson MA, Hall CM, Warner DT, Berger AE, Wenzel AH, Gish DT, Neil GL (1975) Nucleic acids. 14. ara-Cytidine acylates. Use of drug design predictors in structure-activity relation correlation. J Med Chem 18:339–344. doi:10.1021/jm00238a003