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Synthesis of novel 2-(4-(2-morpholinoethoxy)phenyl)-N-phenylacetamide analogues and their antimicrobial study

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Abstract. A new class of potential biologically active 2-(4-(2-morpholinoethoxy)phenyl)-N-phenylacetamides has been synthesized from hydroxyphenylacetic acid. The products were characterized through IR, ^{1}H NMR, mass spectral studies and elemental analysis. The compounds were screened for antimicrobial activity by disc agar diffusion technique. The potency of compounds is tested against variety of fungal and bacterial strains in comparison to clotrimazole and streptomycin, respectively. Some of the synthesized compounds exhibit superior *in vitro* activity compared to the standard drugs.

Keywords. 2-(4-(2-Morpholinoethoxy)phenyl)-N-phenylacetamides; *in vitro* antimicrobial activity; heterocycles; clotrimazole.

1. Introduction

The discovery of penicillin by Alexander Fleming in 1928 is a milestone in the history of medicine.[1] It was predicted that infectious diseases would be completely eliminated by the discovery of more antimicrobials. Unfortunately, the microorganisms are becoming resistant more quickly than new drugs are being made available. The rapid emergence of antibacterial resistance and the lack of novel antibiotics in the past several decades enforce researchers to discover novel antibiotics to combat antimicrobial resistance.[2]

The design of new antimicrobial agents with reduced toxicity and lower side effect is a continuous process. Many heterocycles were frequently encountered in medicinal chemistry whose derivatives represent a class of compounds possessing a wide spectrum of antimicrobial activity. One such heterocycle morpholine is a building block in many pharmaceutical preparations like the antibiotic linezolid,[3] the anticancer agent gefitinib[4] and the analgesic dextromoramide.[5] Certain morpholino acetimidamide derivatives were found to suppress hepatitis C virus replication.[6] Paracetamol, a simple acetamide is a widely used analgesic and antipyretic agent. Acetamide derivatives show potential biological functions. They possess excellent antimicrobial,[7] antiinflammatory,[8] antioxidant,[9] tranquilizer[10] and antagonist properties.[11] In view of this, it was proposed to synthesize novel morpholino acetamides and to study their biological activity.[12]

2. Experimental

2.1 *Materials*

Chemicals used were of analytical grade. The reactions were monitored by TLC on aluminium-backed silica plate visualized by UV-light. Melting points were determined on a Thomas Hoover capillary melting point apparatus using digital thermometer. ^{1}H NMR spectra were recorded on a Bruker 300 MHz spectrometer in $CDCl_3$. Chemical shifts were reported in parts per million down field from tetramethyl silane. Mass spectra were recorded on a VG70-70 H mass spectrometer.

2.2 *Synthesis of 2-(4-hydroxyphenyl)-N-phenyl acetamides*

The synthetic sequence is outlined in scheme 1.[13–17] A mixture of 2-(4-hydroxyphenyl)acetic acid (1.10 mmol) and corresponding amine (**2a**–**e**. 10 mmol) in few mL of water and few drops of pyridine catalyst was stirred for 48 h at room temperature. The product was extracted with ice cold ether (3 × 40 mL). The ether layer was washed with 2% sodium carbonate solution (3 × 20 mL), 2% hydrochloric acid solution (3 × 30 mL) and distilled water. Then the organic layer was dried over

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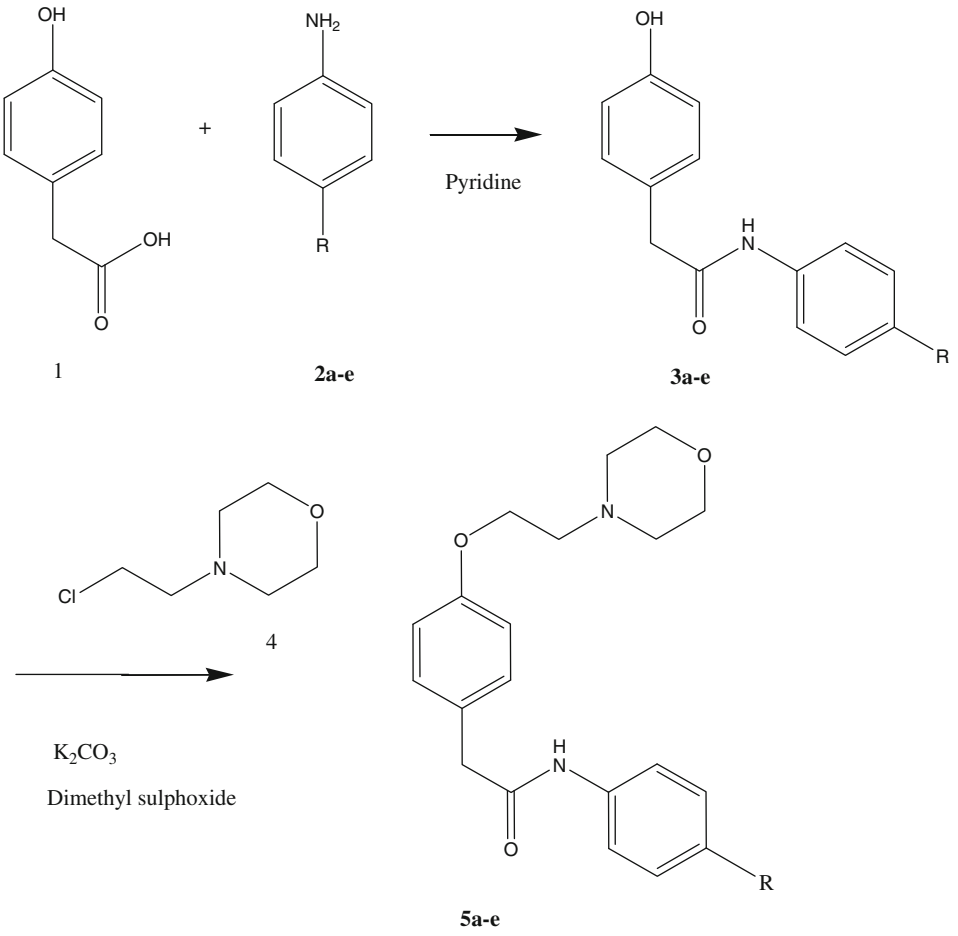

Scheme 1. Synthesis of 2-(4-(2-morpholinoethoxy)phenyl)-N-phenylacetamides.

anhydrous sodium sulphate and evaporated to dryness. The crude product was recrystallized using alcohol to afford 2-(4-hydroxyphenyl)-N-phenyl acetamides (**3a–e**).

2.3 *Charecterisation of compounds (**3a–e**)*

2.3a *Compound **3a**. 2-(4-hydroxyphenyl)-N-phenyl-acetamide*: Mp 160–162°C; IR(Nujol) 1695 cm^{-1} (amide, C=O), 3360 cm^{-1}(amide, N-H), 3525–3610 cm^{-1} (O-H); ^{1}H NMR($CDCl_3$); δ 2.6(s, 2H, CH_2), 3.12(s distd, 1H, N-H), 6.8–7.8(m, 9H, Ar-H), 9.0(br s, 1H, OH); EI-MS; m/z 227 (M^+, 75), 228 (M^+, 84), 226 (100), 121 (43), 93 (78). Anal. Calcd for $C_{14}H_{13}NO_2$ (227); C, 73.98; H, 5.77; N, 6.17. Found; C, 74.02; H, 5.75; N, 6.19.

2.3b *Compound **3b**. 2-(4-hydroxyphenyl)-N-(4-nitro-phenyl)acetamide*: Mp 172–174°C; IR(Nujol) 1725 cm^{-1} (amide, C=O), 3420 cm^{-1} (amide, N-H), 3500–3615 cm^{-1} (O-H); ^{1}H NMR($CDCl_3$); δ 2.62(s, 2H, CH_2), δ 3.1(s distd, 1H, N-H), 7.1–7.8(m, 8H, Ar-H), 9.02(br s, 1H, OH); EI-MS; m/z 272 (M^+, 62), 273 (M^+, 87), 274 (M^+, 45), 139 (62), 104 (32). Ana. Calcd for $C_{14}H_{12}N_2O_4$ (272); C, 61.76; H, 4.44; N, 10.30. Found; C, 61.75; H, 4.42; N, 10.32.

2.3c *Compound **3c**. N-(4-bromophenyl)-2-(4-hydroxy-phenyl)acetamide*: Mp 169–171°C; IR(Nujol) 1705 cm^{-1} (amide, C=O), 3385 cm^{-1} (amide, N-H), 3510–3630 cm^{-1} (O-H); ^{1}H NMR($CDCl_3$); δ 2.62(s, 2H, CH_2), δ 3.14(s distd, 1H, N-H), 7.2–7.8(m, 8H, Ar-H), 9.02(br s, 1H, OH); EI-MS; m/z 306 (M^+, 55), 307 (M^+, 92), 308 (M^+,42), 173 (54), 104 (62), 124.5(78). Anal. Calcd for $C_{14}H_{12}BrNO_2$ (306); C, 54.92; H, 3.95; Br, 26.10; N, 4.58. Found; C, 54.88; H, 3.96; Br, 26.15; N, 4.60.

2.3d *Compound **3d**. N-(4-chlorophenyl)-2-(4-hydroxy-phenyl)acetamide*: Mp 170–172°C; IR(Nujol) 1705 cm^{-1} (amide, C=O), 3390 cm^{-1} (amide, N-H), 3530–3635 cm^{-1} (O-H); ^{1}H NMR($CDCl_3$); δ 2.60(s, 2H, CH_2), δ 3.12(s distd, 1H, N-H), 7.12–7.82(m, 8H,

Ar-H), 9.02(br s, 1H, OH); EI-MS; m/z 261.5 (M^+, 45), 262.5 (M^+, 100), 260.5 (M^+, 35), 128.5 (72), 104(53). Anal. Calcd for $C_{14}H_{12}ClNO_2$ (261.5); C, 64.25; H, 4.62; Cl, 13.56; N, 5.35. Found; C, 64.22; H, 4.61; Cl, 13.54; N, 5.36.

2.3e *Compound **3e**. 2-(4-hydroxyphenyl)-N-(4-methylphenyl)acetamide*: Mp 165–167°C; IR(Nujol) 1700 cm^{-1} (amide, C=O), 3395 cm^{-1} (amide, N-H), 3525–3640 cm^{-1} (O-H); ^1H NMR($CDCl_3$); δ 2.58(s, 2H, CH_2), δ 3.08(s distd, 1H, N-H), 6.9–7.8(m, 8H, Ar-H), 9.02(br s, 1H, OH); EI-MS; m/z 241 (M^+, 67), 242 (M^+, 100), 240 (M^+, 38), 108 (51), 104(65). Anal. Calcd for $C_{15}H_{15}NO_2$ (241); C, 74.67; H, 6.27; N, 5.82. Found; C, 74.64; H, 6.25; N, 5.83.

2.4 *Synthesis of 2-(4-(2-morpholinoethoxy)phenyl)-N-phenylacetamides*

A mixture of **3a–e** (2 mmol) and 1-(2-chloroethyl) morpholine hydrochloride (4.2 mmol) in the presence of anhydrous potassium carbonate (5 mmol) and dimethyl sulphoxide (DMSO) (15 ml) was refluxed for 12 h and then cooled. The residual mass was triturated with ice water to remove potassium carbonate and dimethyl sulphoxide. Then the product was extracted with chloroform (3 × 20 mL). The organic layer was washed with saturated sodium chloride-solution (3 × 20 mL) and 2% sodium hydroxide solution (3 × 20 mL) followed by distilled water and dried over anhydrous sodium sulphate. The crude product obtained by the evaporation of organic layer on recrystallizaton using ethanol afforded the title compounds 2-(4-(2-morpholinoethoxy)phenyl)-N-phenylacetamides (**5a–e**). The compounds **3a–e** and **5a–e** were characterized by IR, ^1H NMR, mass spectral studies and elemental analysis.

2.5 *Charecterization of compounds (**5a–e**)*

2.5a *Compound **5a**. 2-(4-(2-morpholinoethoxy)phenyl)-N-phenylacetamide*: Mp 190–192°C; IR(Nujol) 1698 cm^{-1} (amide, C=O), 3370 cm^{-1}(amide, N-H); ^1H NMR ($CDCl_3$); δ 1.34(t, 4H, ring 2N-CH_2), 2.0(t, 2H, N-CH_2), 2.6(s, 2H, CH_2), 3.12(s distd, 1H, N-H), 3.6(t, 2H, O-CH_2), 4.2(t, 4H, ring 2O-CH_2), 6.8–7.8(m, 9H, Ar-H); EI-MS; m/z 341 (M^+, 68), 342 (M^+, 85), 115 (75), 100(38), Anal. Calcd for $C_{20}H_{24}N_2O_3$ (340); C, 70.56; H, 7.11; N, 8.23. Found; C, 70.54; H, 7.12; N, 8.25.

2.5b *Compound **5b**. 2-(4-(2-morpholinoethoxy)phenyl)-N-(4-nitrophenyl)acetamide*: Mp 203–205°C; IR(Nujol) 1708 cm^{-1} (amide, C=O), 3425 cm^{-1} (amide, N-H); ^1H NMR($CDCl_3$); δ 1.34(t, 4H, ring 2N-CH_2), 2.0(t, 2H, N-CH_2), 2.62(s, 2H, CH_2), 3.1(s distd, 1H, N-H), 3.62(t, 2H, O-CH_2), 4.24(t, 4H, ring 2O-CH_2), 7.1–7.8(m, 8H, Ar-H); EI-MS; m/z 387 (M^+, 72), 388 (M^+, 42), 115 (65), 100 (45), Anal. Calcd for $C_{20}H_{23}N_3O_5$ (385); C, 62.33; H, 6.01; N, 10.90. Found; C, 62.31; H, 6.02; N, 10.92.

2.5c *Compound **5c**. 2-(4-(2-morpholinoethoxy)phenyl)-N-(4-bromophenyl)acetamide*: Mp 195–197°C; IR(Nujol) 1710 cm^{-1} (amide, C=O), 3385 cm^{-1} (amide, N-H); ^1H NMR($CDCl_3$); δ 1.34(t, 4H, ring 2N-CH_2), 2.0(t, 2H, N-CH_2), 2.62(s, 2H, CH_2), δ 3.14(s distd, 1H, N-H), 3.6(t, 2H, O-CH_2), 4.22(t, 4H, ring 2O-CH_2), 7.2–7.8(m, 8H, Ar-H; EI-MS; m/z 420 (M^+, 65), 421 (M^+, 80), 115 (55), 100 (38), Anal. Calcd for $C_{20}H_{23}BrN_2O_3$ (419); C, 57.29; H, 5.53; Br, 19.06; N, 6.68. Found; C, 57.25; H, 5.52; Br, 19.08; N, 6.70.

2.5d *Compound **5d**. 2-(4-(2-morpholinoethoxy)phenyl)-N-(4-chlorophenyl)acetamide*: Mp 198–200°C; IR(Nujol) 1705 cm^{-1} (amide, C=O), 3390 cm^{-1} (amide, N- H); ^1H NMR($CDCl_3$); δ 1.34(t, 4H, ring 2N-CH_2), 2.0(t, 2H, N-CH_2), 2.60(s, 2H, CH_2), δ 3.12(s distd, 1H, N-H), 3.6(t, 2H, O-CH_2), 4.22(t, 4H, ring 2O-CH_2), 7.12–7.82(m, 8H, Ar-H); EI-MS; m/z 375.5 (M^+, 78), 376.5 (M^+, 90), 115 (55), 100 (32), Anal. Calcd for $C_{20}H_{23}ClN_2O_3$ (374.5); C, 60.08; H, 6.18; Cl, 9.46; N, 7.47. Found; C, 60.02; H, 6.16; Cl, 9.44; N, 7.49.

2.5e *Compound **5e**. 2-(4-(2-morpholinoethoxy)phenyl)-N-(4-methylphenyl)acetamide*: Mp 194–196°C; IR(Nujol) 1700 cm^{-1} (amide, C=O), 3395 cm^{-1} (amide, N-H); ^1H NMR($CDCl_3$); δ 1.34(t, 4H, ring 2N-CH_2), 2.0(t, 2H, N-CH_2), 3.58(t, 2H, O-CH_2), 4.18(t, 4H, ring 2O-CH_2), 2.58(s, 2H, CH_2), δ 3.08(s distd, 1H, N-H), 6.9–7.8(m, 8H, Ar-H); EI-MS; m/z 353 (M^+, 70), 356 (M^+, 92), 115 (42), 100 (45), Anal. Calcd for $C_{21}H_{26}N_2O_3$ (354); C, 71.16; H, 7.38; N, 7.90. Found; C, 71.13; H, 7.36; N, 7.92.

2.6 *Antimicrobial study*

The *in vitro* antimicrobial activity of the compounds **1**, **3a–e**, **5a–e** and morpholine were studied by disc agar diffusion technique[18] at different concentrations ranging from 20 to 250 mg/mL using ethylacetate as control. The specific bacterial culture was spread uniformly over nutrient agar in Petri plates. Then the test solution, standard and control of known similar concentrations were spotted in sample wells at specific distance. The zones of inhibition[19] were measured after 24 h

Table 1. Antifungal screening results of compounds **3a–e** and **5a–e** (IC_{50} values in μg/mL).

Compound	*C. albicans*	*C. parapsilosis*	*F. moniliforme*	*A. ochraceus*
3a	70	80	110	70
3b	70	80	90	40
3c	60	70	90	50
3d	60	70	80	70
3e	70	80	100	70
5a	40	70	80	60
5b	20	30	60	20
5c	20	30	70	30
5d	30	40	70	40
5e	40	60	70	50
Control	–	–	–	–
1	80	90	120	70
Morpholine	90	100	120	80
Clotrimazole	30	30	40	20

incubation at 37°C for antibacterial activity and 48 h incubation at 25°C for antifungal activity. The half maximal inhibitory concentration (IC_{50}) is determined for each pathogen and the experiments were carried out in duplicate repeatedly to get reproducible results.

3. Results and discussion

3.1 *Antifungal activity*

The compounds were screened against the fungi *C. albicans*, *C. parapsilosis*, *F. moniliforme* and *A. ochraceus* using clotrimazole as the reference standard and the results are summarized in table 1. The synthesized acetamides **3a–e** possess enhanced activity when compared to morpholine and the compound 2-(4-hydroxyphenyl)acetic acid which is the core structure in the series. The efficacy of title compounds **5a–e** is comparable with that of standard drug clotrimazole. The activity results reveal that the compounds **5b**, **5c** and **5d** possessing nitro, bromo and chloro groups, respectively exhibited maximum activity by inhibiting growth of all the fungi to a significant level in comparison to the standard drug. The compounds **5a** and **5e** are slightly less potent due to absence of active functional groups. All the compounds of the series are more potent to *C. albicans* and *C. parapsilosis* and were less potent to *F. moniliforme* and *A. ochraceus*.

Table 2. Antibacteerial screening results of compounds **3a–e** and **5a–e** (IC_{50} values in μg/mL).

Compound	*P. vulgaris*	*P. fluorescenes*	*S. aureus*	*B. mycoides*
3a	80	70	110	100
3b	60	40	90	90
3c	70	40	90	80
3d	70	50	90	80
3e	80	60	100	100
5a	60	40	80	90
5b	40	30	70	80
5c	40	30	70	60
5d	60	40	80	70
5e	60	40	80	90
Control	–	–	–	–
1	90	90	120	120
Morpholine	120	90	120	150
Streptomycin	50	30	60	70

3.2 *Antibacterial activity*

The screening results of the compounds against the bacteria *P. vulgaris*, *P. fluorescenes*, *S. aureus* and *B. mycoides* along with the standard streptomycin were summarized in table 2. Once again, the synthesized acetamides **3a–e** showed increased potency when compared to morpholine and 2-(4-hydroxyphenyl)acetic acid and the efficacy of title compounds **5a–e** is comparable with that of standard drug streptomycin. The compounds **5a–e** were almost equipotent to *P. vulgaris* and *P. fluorescenes* where as less potent to *S. aureus* and *B. mycoides* when compared to the standard. The compounds **5b**, **5c** and **5d** possess excellent activity against the bacteria. This may be due to the presence of nitro and halo groups on N-phenyl ring.

4. Conclusions

In conclusion, the synthesized compounds **5a–e** have remarkable antifungal and antibacterial activity. The activity of compounds **5b**, **5c** and **5d** with 4-Nitrophenyl and 4-Halophenyl groups indicate the importance of functional groups in enhancing the antimicrobial activity of a compound. The antimicrobial potency of the compounds is more against Gram negative bacteria compared to Gram positive bacteria. The efficacy of compounds has a bright prospect for the discovery of many new drugs used in the treatment of fungal and bacterial infections. Finally, one can conclude that the condensation of two bio-active compounds may be useful to produce new compounds with desired activity.

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