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Synthesis and Antibacterial Activity of Farinomalein and Its Analogues

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ABSTRACT

The present work describes the synthesis and characterization of farinomalein **1** and its analogues. The analogues of farinomalein **1** show significant antibacterial activity against *Proteus vulgaris* and *Staphylococcus aureus*.

KEYWORDS

Maleimides, Stobbe condensation, Haval–Argade contra-thermodynamic rearrangement, Antibacterial activity.

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INTRODUCTION

The compound farinomalein or farinomalein A (**1**) having molecular formula $C_{10}H_{13}NO_4$ (m.w. 211.2145) is first reported by Putri *et al.* [1]. This maleimide-bearing compound is isolated from an entomopathogenic fungus *Paecilomyces farinosus* HF599. The structure of novel farinomalein is evidently determined by the spectroscopic analysis by chemically converting it into its methyl ester of farinomalein **B**. Farinomalein (**1**) has been evaluated for its potent antifungal activity (MIC value 5 µg/disk) against the havoc-generating plant pathogenic *Phytophthora sojae* P6497, wherein the positive control, amphotericin B, used in the assay remarkably shows MIC value of 10 µg/disk [2]. The derivatives of farinomalein, *viz.*, farinomalein **C**, **D** and **E** are isolated from an unidentified endophytic marine-derived fungus. Amrani *et al.* [2] isolated this fungus from the mangrove plant *Avicennia marina* growing in Oman. The structures of the farinomalein **C**, **D** and **E** are supported by optical rotation and spectroscopy method. Miles and Yan [3] reported the first synthesis of farinomalein *via* γ-hydroxybutenolide with 89 % yield. The second improved synthesis of farinomalein **1** has been reported by Aiwale *et al.* [4]. Aiwale and coworkers further extended their work on the synthesis of farinomalein **C–E** [5]. In the literature, farinomalein **A–E** derivatives are reported for their fungicidal activity against plant pathogen *P. sojae* P6497 and *Cladosporium cladosporioides* [6,7]. The naturally occurring farinomalein **1** and derivatives are significant as potent fungicidal agent. This article details on synthesis of farinomalein **1** from isofarinomalein **2** (Fig. 1) and the farinomalein analogues are synthesized from their corresponding alkyl, aryl maleic anhydride as potential candidates bearing antibacterial activity.

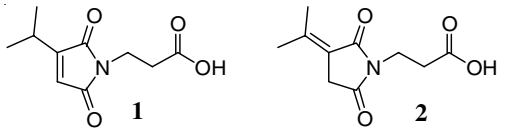


Fig. 1. Structure of farinomalein **1** and isofarinomalein 2 (pseudonym)

EXPERIMENTAL

The solvents used were distilled and dried as per the standard laboratory procedures. Other chemical reagents used were of analytical grade with a minimum purity of 99.5 %. TLC analyses were performed over precoated silica gel 60 F_{245} aluminum plates (Merck®). All melting points are recorded by the open capillary method and are uncorrected. Methanol was used for sample preparation unless stated otherwise. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker® AC-200 and Varian Mercury Spectrometer 400 MHz unless stated otherwise using $CDCl_3$ and CD_3OD as a solvents, chemical shifts are given in parts per million (ppm) with respect to internal standard tetramethylsilane (δ_H 4.78 and 3.31 and δ_C 49.1 in CD_3OD) and coupling constant *J* values are quoted in Hertz. The HOD (δ_H 1.5) peak was ignored from the spectrum in case of hygroscopic compounds. Mass spectra were recorded on Shimadzu® GCMS-QP5050A spectrometer or Agilent® G6540B MS Q-TOF at 70 eV with the electron spray ionization technique. The bacterial culture of *Staphylococcus aureus* (Gram-positive) (NCIM No. 2079, ATCC No. 6538P) and *Proteus vulgaris* (Gram-negative) (NCIM No. 2813, ATCC No. 9484) strain was procured from NCIM, National Chemical Laboratory, Pune-08. Nutrient dehydrated powder and agar agar were purchased from Hi-Media®.

3-(Methoxycarbonyl)-4-methylpent-3-enoic acid (3): To a solution of sodium methoxide, prepared from sodium metal (3.145 g, 136.85 mmol) and methanol (50 mL), were added dimethyl succinate **4** (5.00 g, 34.21 mmol) and acetone (2 g, 34.22 mmol) under nitrogen [8]. The mixture was refluxed for 6 h, poured into stirred, aqueous HCl (2 N, 85 mL) at 0 °C and extracted with ethyl acetate. The extract was washed with water and was extracted with saturated sodium bicarbonate (150 mL). The aqueous phase was separated, washed with ethyl acetate and acidified with aqueous HCl (10 %). The aqueous solution was extracted with ethyl acetate and the extract was washed with water and brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by silica gel column chromatography using ethyl acetate and hexane (9:1), which gave 3-(methoxycarbonyl)-4-methylpent-3-enoic acid **3** (4.56 g, 77 %) as pale yellow liquid. Thin-layer chromatography was performed using chloroform (2 runs), R_f: 0.34 at $UV_{254\ nm}$. Yield: 77 %. IR (KBr, ν_{max}, cm^{-1}): 3529, 2956, 2645, 1720 (broad), 1643, 1437, 1371, 1204, 1179, 1085, 922, 803. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.746 (3H, *s*), 3.421 (2H, *s*), 2.163 (3H, *s*), 1.898 (3H, *s*). HRMS: Calculated for $C_8H_{12}O_4$ M^+ 172.0736, Found 172.0738.

3-[2,5-Dioxo-3-(propan-2-ylidene)pyrrolidin-1-yl]-propanoic acid [isofarinomalein (2)]: To a stirred solution of 3-(methoxycarbonyl)-4-methylpent-3-enoic acid (**3**) (0.500 g, 2.9 mmol) and in 5 mL glacial acetic at room temperature was added β-alanine (0.310 g, 3.5 mmol) with nitrogen. The reaction mixture was heated to reflux for 2 h and then cooled at 60 °C and the solvent was evaporated under vacuum. Ethyl acetate (100 mL) was added to the crude product and then the organic layer was washed with 2 N HCl followed by water and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product obtained was purified by silica gel column chromatography, which gave 3-[2,5-dioxo-3-(propan-2-ylidene)pyrrolidin-1-yl]propanoic acid [isofarinomalein (**2**)] (0.417 g, 68 %) (m.p.: 190–192 °C). Thin-layer chromatography was performed using ethyl acetate: hexane (50:50), R_f: 0.29 at $UV_{254\ nm}$. IR (KBr, ν_{max}, cm^{-1}): 3425, 2971, 2926, 1868, 1785, 1421, 1210, 1057, 917. ^{1}H NMR (500 MHz, $CDCl_3$): d 3.87 (2H, *t*, *J* = 7 Hz), 3.23 (2H, *s*), 2.36 (3H, *s*), 2.70 (2H, *t*, *J* = 7 Hz), 1.90 (3H, *s*). ^{13}C NMR (50 MHz, $CDCl_3$): d 175.65, 173.86, 169.27, 150.30, 118.59, 33.92, 33.62, 31.79, 24.11, 20.63. LC MS-MS: $C_{10}H_{13}NO_4$ *m/z* (intensity) 194.08117 (20), 166.08626 (8), 152.0.698 (100), 140.07061 (18), 124.07569 (34), 95.04914 (13), 67.05423 (5) and 55.01839 (99).

Farinomalein (**1**): The compound isofarinomalein **2** (0.150 g, 0.71 mmol) was subjected to the Haval–Argade contra-thermodynamic rearrangement [9], which involves three steps. The mixture of compounds was obtained from sequence of three steps in (0.044 g, 33 %) yields. The ratio (14.6:85.4) of farinomalein **1**:isofarinomalein **2** was calculated from NMR spectra. The structure of the compound was supported by LC-MS/MS Q-TOF and its fragmentation pattern. Thin-layer chromatography was performed using diethyl ether:hexane (50:50), R_f: 0.32 at $UV_{254\ nm}$. ^{1}H NMR (500 MHz, $CDCl_3$): δ 9.43 (1H, *bs*), 6.62 (1H, *s*), 3.73 (2H, *t*, *J* = 7.5 Hz), 2.78 (1H, *sept*, *J* = 7 Hz), 2.64 (2H, *t*, *J* = 7.5 Hz) and 1.28 (6H, *d*, *J* = 7 Hz). LC MS-MS: $C_{10}H_{13}NO_4$ *m/z* (intensity) 194.0793 (37), 178.08626 (3), 166.0854 (100), 152.07061 (34), 134.06004 (20), 124.0745 (23), 106.0644 (76), 94.06513 (53), 79.05423 (69), 73.0540 (15), 67.5423 (20), 55.01784 (32), 45.03349 (22) and 41.03858 (12).

General procedure for preparation of maleimide from corresponding anhydride

3-[2,5-dioxo-2*H*-pyrrol-1(5*H*)-yl)]propanoic acid (5a): To a solution of (1.000 g, 10.20 mmol) of maleic anhydride **5** in 5 mL glacial acetic acid at room temperature was added β-alanine (1.090 g, 12.23 mmol) with nitrogen. The reaction mixture was heated to reflux for 2 h and then cooled at 60 °C and the solvent was evaporated under vacuum. Ethyl acetate (100 mL) was added to the crude product and then the organic layer was washed with 2 N HCl followed by water and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product **5a** obtained was purified by silica gel column chromatography afford (1.115 g, 65 %) as white solid (m.p.: 103–106 °C). Thin layer chromatography was performed using ethyl acetate: hexane (50:50), R_f: 0.55 at $UV_{254\ nm}$. ^{1}H NMR (200 MHz, CD_3OD): δ 6.82 (2H, *s*), 3.76 (2H, *t*, *J* = 6 Hz), 2.59 (2H, *t*, *J* = 6 Hz). IR (KBr ν_{max}, cm^{-1}): 3094, 2959, 1706, 1640, 1413, 1231, 1152, 833, 696. LC MS-MS: $C_7H_7NO_4$ *m/z* (intensity) 170.0447 (36), 152.0337 (100), 150.0233 (30), 142.0497 (43), 139.0562 (7), 130.0492 (3), 128.03339 (4), 124.0388 (4), 113.232 (36), 110.0233 (24), 105.0339 (4), 100.0395 (120), 98.0238 (8), 87.0441 (6), 72.0447 (9) and 56.0180 (51).

3-[3-Methyl-2,5-dioxo-2*H*-pyrrol-1(5*H*)-yl)]propanoic acid (6a): Thin-layer chromatography was performed using ethyl acetate: hexane (50:50), R_f: 0.25 at $UV_{254\,nm}$. Yield: 64 %, white, crystalline (m.p.: 88–90 °C). IR (KBr, ν_{max}, cm^{-1}): 3104, 2954, 2643, 1723, 1709, 1696, 1641, 1443, 1410, 1333, 1293, 1233, 1131, 1104, 944 and 871. ^{1}H NMR (200 MHz, CD_3OD): δ 6.33 (1H, *s*), 3.79 (2H, *d*, *J* = 6 Hz), 2.67(2H, *d*, *J* = 6 Hz), 2.08 (3H, *s*). LC MS-MS: $C_8H_9NO_4$ *m/z* (intensity) 184.0603 (45), 166.0493 (93), 156.0050 (11), 142.0496 (100), 134.0077 (23), 124.0392 (94), 113.9756 (10), 105.0327 (10), 100.1116 (16), 88.0581 (10), 69.0704 (8), 58.0559 (17) and 55.0175 (26).

3-[2,5-Dioxo-3-phenyl-2*H*-pyrrol-1(5*H*)-yl)]propanoic acid (7a): Thin-layer chromatography was performed using ethyl acetate: hexane (50:50), R_f: 0.24 at $UV_{254\,nm}$. Yield: 73 %, white, crystalline solid (m.p.: 132–135 °C). IR (KBr, ν_{max}, cm^{-1}): 3449, 3096, 2941, 1764, 1717, 1699, 1609, 1396, 1232, 938 and 790. ^{1}H NMR (200 MHz, DMSO-d_6): δ 12.40 (1H, *bs*), 8.02 (2H, *d*, *J* = 8 Hz), 7.99 (1H, *t*, *J* = 8 Hz), 7.51 (2H, *m*, *J* = 8 Hz), 7.38 (1H, *s*), 3.69 (2H, *t*, *J* = 6 Hz), 2.55 (2H, *t*, *J* = 6 Hz). LC MS-MS: $C_{13}H_{11}NO_4$ *m/z* (intensity) 246.0749 (11), 228.0655(83), 150.0233 (30), 186.0547 (100), 98.09643 (2) and 55.0177 (5).

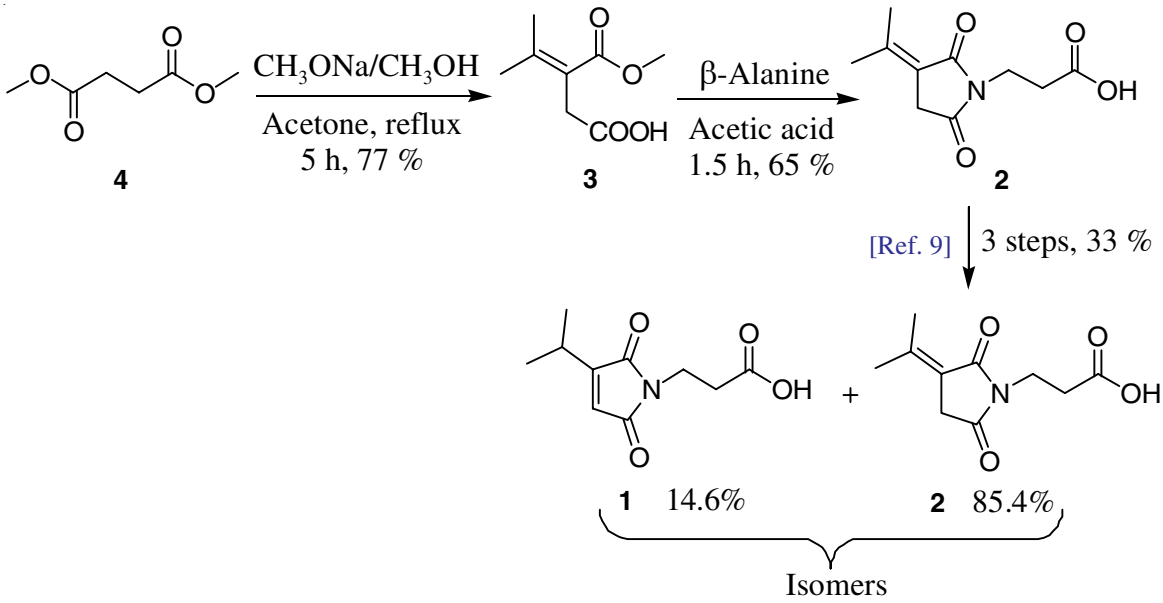
3-(1,3-Dioxoisoindolin-2-yl)propanoic acid (8a): Thin-layer chromatography was performed using ethyl acetate: hexane (50:50), R_f: 0.29 at $UV_{254\,nm}$. Yield: 92 %, white, crystalline solid (m.p.: 151–153 °C), IR (KBr, ν_{max}, cm^{-1}): 3206, 1777, 1718, 1698, 1438, 1407, 1357, 1253, 1212, 999, 822 and 725. ^{1}H NMR (200 MHz, CD_3OD): δ 7.83 (2H, *d*, *J* = 8 Hz), 7.73 (2H, *d*, *J* = 8 Hz), 4.01 (3H, *d*, *J* = 6 Hz), 2.80 (3H, *d*, *J* = 6 Hz). LC MS-MS: $C_{11}H_9NO_4$ *m/z* (intensity) 220.0604 (7), 202.0497 (100), 188.9865 (2), 180.0390 (85), 119.0868 (4), 93.0687 (4), 81.0671 (3) and 55.0182 (2).

3-(2,5-Dioxopyrrolidin-1-yl)propanoic acid (9a): Thin-layer chromatography was performed using dichloromethane: acetone: acetic acid 79:20:1, R_f: 0.28 (reported 0.20), crystalline, white solid (m.p.: 158–160 °C), Yield: 70 %. IR (KBr, ν_{max}, cm^{-1}): 3001, 2963, 2707, 2601, 2494, 1773, 1723, 1669, 1450, 1417, 1400, 1345, 1307, 1259, 1193, 1165, 1081, 999, 909, 818, 775 and 699. HRMS: Calculated for $C_7H_8NO_4Na$ $[M + Na]^+$ 193.0351, Found 193.0389.

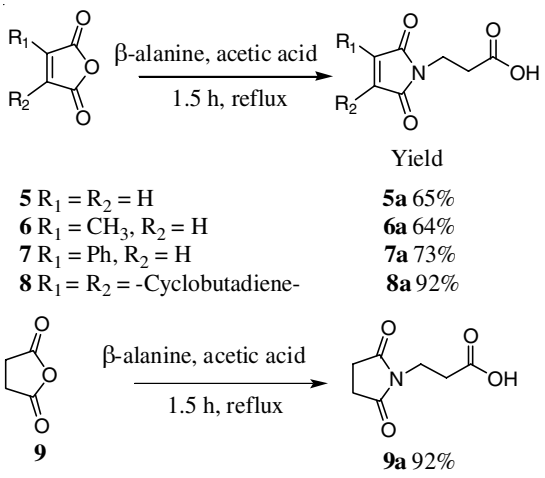
RESULTS AND DISCUSSION

The dimethyl succinate **4** and acetone refluxed with the alcoholic sodium methoxide solution rendered compound **3** with 77 % yield (**Scheme-I**). The molecular formula of **3** was confirmed as $C_8H_{12}O_4$ by HRMS. The novel compound, isofarinomalein **2**, was prepared by the simple condensation method and the structure was confirmed by ^{1}H NMR, ^{13}C NMR and LC-MS/MS data. Isofarinomalein **2** was subjected to the Haval–Argade contra-thermodynamic rearrangement involving three steps [9]. The yield of the mixture obtained from this sequence was as low as 33 %. Farinomalein **1** and isofarinomalein **2** were found in ratio 14.6:85.4. This ratio was deduced from the integration of protons observed in ^{1}H NMR spectra of the mixture. The structures of the compounds **1** and **2** were confirmed by the LC-MS/MS fragmentation pattern. The compounds **5a-9a** were synthesized from commercially available substituted maleic anhydride and succinic anhydride (**Scheme-II**). The structures of these analogues were certainly confirmed with the help of physical constant, ^{1}H NMR and HRMS.

The antibacterial activity, structure–activity relationship, of farinomalein analogues was evaluated against *P. vulgaris* and *S. aureus* using the zone inhibition method. The DMSO was used as a negative control and all observations were recorded in triplicate. The compounds **2, 5a, 8a, 6a** and **7a** were showed 8 ± 0.6, 8 ± 0.6, 8 ± 0.6, 11 ± 0.6 and 21 ± 1.0 zone of inhibition in mm respectively against *P. vulgaris* (Fig. 2). The compound **7a** recorded with maximum zone of inhibition, whereas compound **9a** showed no activity against *P. vulgaris*. Maleimide was assumed to be a model scaffold compound within the series of farinomalein analogues observed with moderate antibacterial activity. The compounds **2** and **7a** were showed 10 ± 0.0 and 16 ± 1.2 zone of inhibition in mm respectively against *S. aureus*



Scheme-I: Synthesis of farinomalein **1**



Scheme-II: Preparation of farinomalein analogues **5a-9a**

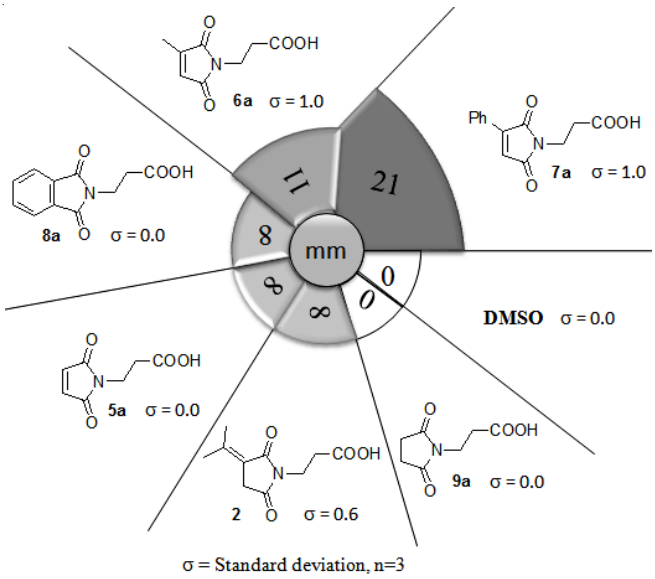


Fig. 2. Plot of zone of inhibition (mm) *versus* structure, at concentration 1 mg/mL against clinical pathogen *P. vulgaris*

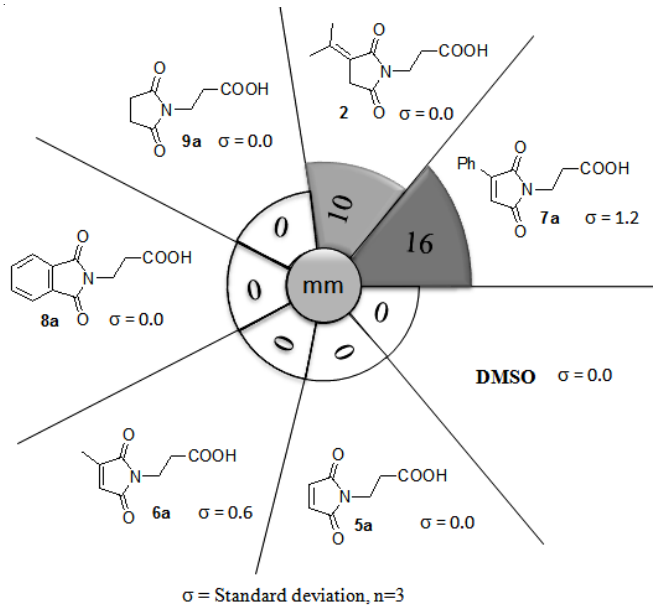


Fig. 3. Plot of zone of inhibition (mm) *versus* structure, at concentration 1 mg/mL against clinical pathogen *S. aureus*

(Fig. 3). The compound **7a** recorded with maximum zone of inhibition, whereas compounds **5a, 6a, 8a** and **9a** showed no activity against *S. aureus*. Empirically, phenyl analogue of farinomalein **7a** was found with broad-spectrum activity and a promising compound among the series of other compounds is studied in this scheme.

Conclusion

The Stobbe condensation followed by the Haval–Argade contra-thermodynamic rearrangement strategy was originally deployed for synthesis of farinomalein; however, the yield of the reactions involved was insignificant. Practically, phenyl analogues of farinomalein **1** and isofarinomalein **2** were found with significant broad-spectrum antibacterial activity among other analogues of farinomalein against *P. vulgaris* and *S. aureus*.

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