Phytotoxic Arylethylamides from Limnic Bacteria using a Screening with Microalgae‡

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N-Phenylethylamides **1a - 1f**, were isolated from cultures of three limnic strains GW90a, GW102a and GW73a. Strain GW102a delivered additionally the compound *cyclo*(isoleucyldehydroalanyl) (**2**). The structure of these compounds were assigned by a detailed spectral analysis. Due to their potential use as herbicides, various related compounds **1a**, **3**, **4a** and **4b** were synthesized. The minimum inhibitory concentration (MIC) against *Chlorella vulgaris, Chlorella sorokiniana, Chlorella salina* and *Scenedesmus subspicatus* ranged from 100 to 12.5 μg/ml. All these amides were found to be inactive against *Mucor miehei*, *Candida albicans*, and some bacteria.

Aquatic microorganisms are a rich source of new and biomedically relevant constituents[1)]. We have focussed our efforts to explore the potential of biologically active marine and limnic bacterial extracts for antimicroalgal, antibacterial and antifungal activity[2)]. In the course of

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this screening, extracts of the limnic strains GW90a, GW102a and GW73a were shown to possess potent antimicroalgal activity.

Fermentation of strain GW90a and separation of the extract afforded four new N-phenylethylamides **1b - 1e**. From the strain GW102a in addition to the metabolites above, the new compounds 2-methyl-N-(2'-phenylethyl)butyramide (**1f**) and *cyclo*(isoleucyldehydroalanyl) (**2**) were isolated, while another limnic strain GW73a delivered the arylethylamide **1d**. Due to their expected biological properties, arylethylamides **1a** and **3** as well as their analogues **4a** and **4b** were synthesized. This paper deals with the taxonomic characterisation of the producing organisms, the production, isolation and synthesis of arylethylamides and analogues, and describes their antimicroalgal activity.

Taxonomic Studies of Producing Strain

The strains GW73a, GW90a, and GW102b were enriched from a sediment sample from the waste storage site Georgswerder close to Hamburg, Germany, and were able to grow on a minimal medium containing biphenyl as the sole carbon source.

The strains were investigated by FAME (fatty acid methyl ester) analysis and shown to belong to a tight cluster with an Euclidean distance <10. On the basis of the FAME profiles, the strains were assigned to the *Nocardiopsis* group of organisms but could not be identified further. Sequencing of the 16S rDNA showed GW73a to be most similar but not identical to *Bacillus cereus* (98 % sequence identity), GW90a to an unknown *Bacillus* strain (96 % sequence identity) and GW102b to *Bacillus thuringiensis* (96 % sequence identity). Thus, these strains probably represent new species or subspecies within the genus *Bacillus*.

Fermentation and Isolation

The producing limnic strains GW90a, GW102a, GW73a were inoculated from agar culture into Erlenmeyer flasks with Luria-Bertani medium and incubated for 3 days at 28 °C. Upscaling was done in 20 l jar fermentors under similar conditions.

The ethyl acetate extract, obtained after work-up of the culture, was defatted with cyclohexane and subjected to silica gel column chromatography to separate various fractions.

((insert Figure 1. here: Work-up of the strain GW90a))

Activity screening of the fractions was done by agar diffusion tests using the algae *Chlorella vulgaris*, *Chlorella sorokiniana*, *Chlorella salina*, and *Scenedesmus subspicatus*. The active fractions were purified by HPLC and Sephadex LH 20 to afford six new colourless compounds namely N-(2'-phenylethyl)propionamide (**1b**), N-(2'-phenylethyl)isobutyramide (**1c**), 3-methyl-N-(2'-phenylethyl)butyramide (**1d**), N-(2'-phenylethyl)hexanamide (**1e**), and 2-methyl-N-(2'-phenylethyl)butyramide (**1f**) along with the known compounds *cyclo*(isoleucyldehydroalanyl) (**2**), N-(2'-phenylethyl)acetamide (**1a**), N_β-acetyltryptamine, *cyclo*(tyrosylprolyl), *cyclo*(leucylprolyl), uridine, uracil, and anthranilic acid. All these compounds were identified by IR, ^{1}H NMR, ^{13}C NMR and EI-MS/CI-MS and by comparison with data from AntiBase[3)].

Results and Discussion

The molecular formula of **1b** was determined to be $C_{11}H_{15}NO$ by HR-MS of the molecular ion at *m*/*z* 177.1. The IR spectrum showed bands at 3275 and 1648 cm^{-1}, pointing to the presence of an amide group. The ^{1}H and ^{13}C NMR data confirmed the presence of an amide carbonyl and indicated three methylenes, one methyl and a phenyl group. Based on the spectroscopic information and the comparison of NMR data with the known amide **1a**, the structure was assigned as N-(2'-phenylethyl)propionamide (**1b**) and finally confirmed by synthesis.

The DCI mass spectra of three further isolated compounds led to the molecular weights of 191, 205 and 219 Dalton, respectively. The ^{1}H NMR spectra showed an phenylethyl system as in **1b** with the variation in the signals for the acid, which suggested these metabolites to be phenylethylamides of homologous acids. The spectroscopic data of these compounds led to N-(2'-phenylethyl)isobutyramide (**1c**), 3-methyl-N-(2'-phenylethyl)butyramide (**1d**) and N-(2'-phenylethyl)hexanamide (**1e**).

The CI and EI mass spectra revealed for another compound a molecular weight (205 Dalton) identical with that of **1d**. The ^{1}H NMR spectrum showed it to be also a phenylethylamide, the acid part contributing a sextet at δ 2.01 (1 H), two multiplets at δ 1.63 and 1.39 (1 H each), a doublet at δ 1.07 (J = 8 Hz, 3 H) and a triplet at δ 0.86 (J = 8 Hz, 3 H).

The final structure was assigned by aid of the H,H COSY spectrum as 2-methyl-N-(2'-phenylethyl)butyramide (**1f**) and also confirmed by synthesis.

((insert Table 1 here))

((insert Formula 1a-1f here))

The ^{1}H NMR spectrum of the compound **3** showed two broad D_2O exchangeable signals at δ 10.44 (1 H) and 8.38 (1 H) for NH and/or OH groups. The signals at δ 5.12 (1 H) and 4.78 (1 H) were attributed to olefinic protons. A triplet of doublet at δ 3.96 for a methine proton connected either with a nitrogen or an oxygen atom, a 1H multiplet at δ 1.80, a doublet of triplet (2 H) at δ 1.56 and a doublet for six protons at δ 0.86 (isopropyl group) were observed at the aliphatic region. The ^{13}C NMR and APT spectrum of this compound showed eight signals. The signals at δ 166.4 and 158.2 were interpreted as carbonyl signal of carboxylic acids, amides or esters. The signals at δ 134.6 and 98.9 represent a $C=CH_2$ fragment in conjugation with a carbonyl group, while the signals at δ 22.1 were accounted for the two methyl group of an isopropyl residue. The DCI mass spectrum suggested the molecular weight to be 182 Dalton, which further indicated the molecular formula $C_9H_{14}N_2O_2$. Finally the structure of the compound was assigned by $^{1}H,^{1}H$-COSY, HMQC and HMBC couplings as *cyclo*(isoleucyldehydroalanyl) (**2**).

((insert Figure 2 here))

A piperazinedione with the structure of **2** has been described recently[4]. The NMR data of both compounds, however, differ substantially in the splitting pattern and chemical shift of the methyl groups, although no diastereomers are possible.

In order to study the structure-activity relationship, the N-(2'-phenylethyl)amide **3** and N-(1'-phenylethyl)amides **4a** and **4b** were prepared from the corresponding N-phenylethyl amines and acid chlorides according to the literature and characterized by IR, mass, ^{1}H, and ^{13}C NMR spectra.

((insert Formula 2-4 here))

Antimicroalgal Activity

Antimicroalgal activities were determined using the agar diffusion method and media as described previously[2]. Tab. 1 shows the antimicroalgal activities of compounds **1b**-**1f**, **3**, **4a** and **4b**.

The results of the agar diffusion tests indicated that the phytotoxicity of the phenylethylamides depends strongly on the lipophilicity of the acid residue of the molecule. That might be the reason for the amides of smaller acids being inactive. The (2'-phenylethyl)hexadecanamide, however, was found to be inactive in the agar diffusion test against all three tested micro alga, perhaps due to poor solubility.

For MIC values, liquid medium was inoculated with test organisms and pre-incubated at 24-26 °C for one day in daylight. Test substances were added and results were recorded after 3 days (see Table 2). In addition, a known antibiotic, actinomycin D (AD) showed strong antimicroalgal activity and hence this was tested as a reference.

((insert Table 2 here))

((insert Table 3 here))

Compounds **1b**-**1f**, **3**, **4a**-**4b** did not show activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Streptomyces viridochromogenes*, *Candida albicans* and *Mucor miehei* at concentrations up to 200 μg/ml. The activity of the phenylethylamides is weaker than that of the previously described anthranilamides[2].

Experimental

Material & methods and biological tests were used as described earlier[2].

Isolation of the strains GW73a, GW90a, and GW102b

A sample of ca. 50 ml was collected manually from a seepage channel and contained 15 mg PCB kg^{-1} (dry weight). Slurry microcosms were set up as described[5] by mixing 2 g of the sample with 18 ml of M9 minimal medium and adding biphenyl crystals to yield a final concentration of ca. 650 μg l^{-1} in the liquid phase. Slurries were shaken gently for 6 months on a rotatory shaker. Evaporated water and consumed biphenyl crystals were periodically replaced. An aliquot of 100 μl was serially diluted in 0.85 % (wt/vol) NaCl, and appropriate dilutions were spread on agar plates containing 0.1 × Luria-Bertani (LB) medium[6]. Colonies

which showed activity for the 2,3-dihydroxybiphenyl dioxygenase enzyme (spray test described in Lit.[5]) were picked and subcultivated on 0.1 strength LB plates.

The strains are deposited in the culture collection of the Department of Microbiology at the National Research Institute for Biotechnology in Braunschweig, Germany.

Algae test plates

Strains of *Chlorella vulgaris* (SAG 211-11b), *Chlorella sorokiniana* (SAG 211-8k) and *Scenedesmus subspicatus* (SAG 86-81) were obtained from the Collection of Algae Cultures, Göttingen, Germany (SAG) and cultured in Bold's basal medium (BBM, 1)[7] modified by adding sterile-filtered $NaHCO_3$ (50 mM final concentration). The algae were grown at 20 °C and illuminated by white fluorescent tubes [80 µmol photons m^{-2} s^{-1}, 14/10 h light-dark cycle] or daylight. Algae were harvested for testing after 10 - 14 days at a cell density between 10^7 - 10^8 cells/ml.

The harvested algae were washed and resuspended in Bold's basal medium and were set at a cell density of 5×10^7 cells/ml. 2.5 ml of the algae were added to 2.5 ml of 1.5 % agar in BBM [MBBM, 2] (55 - 60 °C) and immediately poured on Petri dishes containing 15 ml BBM [MBBM] plus 1.5 % agar. The plates were incubated at room temperature for 24 h and then used for the biological assay.

Fermentation of the strains GW90a, GW102a and GW73a

The strains GW90a, GW102a and GW73a were separately inoculated from slant agar culture into 10 × 1 l Erlenmeyer flasks each with 200 ml of Luria-Bertani[6] medium and grown for 3 days at 29 °C with 95 rpm. The shaken cultures of each strain served separately for the inoculation of 20 l jar fermentors each containing 18 l of the medium as above. Incubation was carried out for 3 days at 29 °C and 120 rpm with automatic addition of 2N NaOH and 2N HCl to maintain the pH at 6.5 ± 1.25. Niax was used as antifoaming agent and sterile air (5 l/min) was supplied. The culture broth of each fermentor was mixed with diatom earth (ca. 1 kg) and passed through a pressure filter. The culture filtrate and biomass were extracted separately three times with ca. 10 l of ethyl acetate and the combined organic layers

were evaporated to dryness to yield 4.12 g (GW90a), 2.40 g (GW102a) and 2.79 g (GW73a), respectively, of crude extracts.

The crude extracts were dissolved in methanol (ca. 100 ml) and defatted with cyclohexane (ca. 100 ml). The methanol layers were concentrated, the residues were dried *in vacuo* and subjected to silica gel column chromatography (65 × 3 cm) using $CHCl_3/CH_3OH$ (GW90a and GW102a) and ethyl acetate/cyclohexane gradient to separate into 10 (GW90a) or 8 (GW102a and GW73a) fractions. Purification of the phytotoxic fraction 4 (GW90a) using HPLC, afforded three colourless compounds N-(2'-phenylethyl)propionamide (**1b**) (19 mg), N-(2'-phenylethyl)isobutyramide (**1c**) (25 mg), 3-methyl-N-(2'-phenylethyl)butyramide (**1d**) (24 mg). Fraction 5 was purified on Sephadex LH 20 ($CHCl_3/CH_3OH$ 6 : 4) and delivered N-(2'-phenylethyl)propionamide (**1b**) (15 mg) and N-(2'-phenylethyl)hexanamide (**1e**) (35 mg). Further purification of the fractions from GW102a afforded **1a**-**1e**, **1f** (6 mg) and **2** (2 mg). Separation of GW73a gave **1d** (14 mg) and the known compounds N-(2-phenylethyl)acetamide (**1a**, 34 mg), N_β-acetyltryptamine (30 mg), *cyclo*(tyrosylprolyl) (66 mg), *cyclo*(leucylproplyl) (23 mg), uridine (9 mg), uracil (3 mg), and anthranilic acid (7 mg).

N-(2'-Phenylethyl)propionamide (**1b**)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (5H, m, Ar-H), 5.50 (1H, br s, exchangeable with D_2O, N-H), 3.52 (2H, q, J = 8 Hz, 1′-H_2), 2.82 (2H, t, J = 8 Hz, 2′-H_2), 2.18 (2H, q, J = 8 Hz, 2-H_2), 1.10 (3H, t, J = 8 Hz, 3-H_3). – ^{1}H NMR (acetone-d_6, 200 MHz) δ 7.24 (5H, m, Ar-H), 7.10 (1H, br s, exchangeable with D_2O, N-H), 3.40 (2H, q, ^3J = 8.0 Hz, 1′-H_2), 2.77 (2H, t, J = 8, 2′-H_2), 2.13 (2H, q, J = 7.0 Hz, 2-H_2), 1.04 (3H, t, J = 7.0 Hz, 3-H_3). – ^{13}C NMR (acetone-d_6, 50.3 MHz) δ 174.0 (C_q-1), 140.5 (C_q-1"), 129.5 (CH-3", 5"-CH), 129.1 (CH-2", CH-6"), 126.9 (CH-4"), 41.4 (CH_2-2), 41.3 (CH_2-1′), 36.5 (CH_2-2′), 10.2 (CH_3-3). – EI-MS (70 eV) *m/z* (%) 177 (M, 68), 104 (Ph-CH_2CH_2, 100). – CI-MS (NH_3) *m/z* (%) 372 ($[2M + NH_4]^+$, 38), 355 ($[2M + H]^+$, 100), 195 ($[M + NH_4]^+$, 70), 178 ($[M + H]^+$, 38). – IR (KBr) ν_{max} (cm^{-1}) 3276, 3080, 3028, 2970, 2934, 1648, 1558, 1496, 1454, 1430, 1375, 1248, 1198, 1132, 1049, 885, 749, 701, 573, 497, 466.

N-(2'-Phenylethyl)isobutyramide (**1c**)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (5H, m, Ar-H), 5.50 (1H, br s, exchangeable with D_2O, N-H), 3.50 (2H, q, J = 8 Hz, 1′-H_2), 2.82 (2H, t, J = 8 Hz, 2′-H_2), 2.30 (1H, h, J = 8 Hz, 2-H), 1.15 (6 H, d, J = 8 Hz, 3-H_3, 4-H_3). – ^{1}H NMR (acetone-d_6, 200 MHz) δ 7.24 (5H, m, Ph-H), 7.04 (1H, br s, exchangeable with D_2O, N-H), 3.40 (2H, q, ^3J = 8.0 Hz, 1′-H_2), 2.77 (2H, t, J = 8.0 Hz, 2′-H_2), 2.36 (1H, h, J = 8.0 Hz, 2-H), 1.04 (6H, d, J = 8.0 Hz, 3-H_3, 4-H_3). – ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 174.0 (C_q-1), 140.5 (C_q-1"), 128.8 (CH-3", CH-5"), 128.6 (CH-2", CH-6"), 126.5 (CH-4"), 40.5 (CH_2-1′), 35.7 (CH_2-2′), 35.6 (CH-2), 19.6 (CH_3-2, CH_3-4). – ^{13}C NMR (acetone-d_6, 50.3 MHz) δ 176.7 (C_q-1), 140.5 (C_q-1"), 129.6 (CH-3", CH-5"), 129.1 (CH-2", CH-6"), 126.9 (CH-4"), 41.3 (CH_2-1′), 36.5 (CH_2-2′), 35.6 (CH-2), 19.9 (CH_3-3, CH_3-4). – EI-MS (70 eV) *m/z* (%) 191.1 (M, 44), 104.0 (Ph-CH_2CH_2, 100), 71.0 (40). – IR (KBr) ν_{max} (cm^{-1}) 3300, 3086, 2967, 2871, 1641, 1548, 1458, 1363, 1242, 1195, 1101, 748, 698, 486.

N-(2'-Phenylethyl)isovaleramide (**1d**)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.28 (5H, m, Ar-H), 5.56 (1H, br s, exchangeable with D_2O, N-H), 3.56 (2H, q, J = 8 Hz, 1′-H_2), 2.82 (2H, t, J = 8 Hz, 2′-H_2), 2.06 (1H, s, J = 8 Hz, 3-H), 1.98 (2H, d, J = 8 Hz, 2-H_2), 0.92 (6H, d, J = 8 Hz, 4-H_3, 5-H_3). – ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 172.9 (C_q-1), 138.9 (C_q-1"), 128.7 (CH-3", CH-5"), 128.6 (CH-2", CH-6"), 126.5 (CH-4"), 46.1 (CH_2-2), 40.6 (CH_2-1′), 35.7 (CH_2-2′), 26.2 (CH-3), 22.4 (CH_3-4, CH_3-5). – CI-MS (NH_3) *m/z* (%) 206.1 $([M + H]^+$, 28), 223.1 $([M + H]^+$, 100), 240.1 $([M + NH_3 + NH_4]^+$, 15), 411.1 $([2M + H]^+$, 28), 428.1 $([2M + NH_4 + NH_3]^+$, 42). – IR (KBr) ν_{max} (cm^{-1}) 3302, 2959, 2868, 1639, 1545, 1456, 1368, 1308, 1198, 1131, 1031, 748, 699, 605, 496.

N-(2'-Phenylethyl)hexanamide (**1e**)

^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.24 (5H, m, Ar-H), 5.48 (1H, br s, exchangeable with D_2O, N-H), 3.50 (2H, q, J = 8 Hz, 1′-H_2), 2.80 (2H, t, J = 8 Hz, 2′-H_2), 2.10 (2H, t, J = 8 Hz, 2-H_2), 1.60 (2H, q, J = 8 Hz, 3-H_2), 1.28 (4H, m, 4-H_2, 5-H_2), 0.85 (3H, t, J = 8 Hz, 6-H_3). – ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 173.3 (C_q-1), 138.5 (C_q-1"), 128.7 (CH-3", CH-5"), 128.6 (CH-2", CH-6"), 126.5 (CH-4"), 40.6 (CH_2-1′), 36.7 (CH_2-2), 35.7 (CH_2-2′), 31.4 (CH_2-3), 26.5

(CH_2-4), 22.4 (CH_2-5), 13.9 (CH_3-6). – CI-MS (NH_3) *m/z* (%) 220 ($[M + H]^+$, 32), 237 ($[M + NH_4]^+$, 100), 439 ($[2 M + H]^+$, 20), 456 ($[2 M + NH_4]^+$, 18). – IR (KBr) ν_{max} (cm^{-1}) 3303, 2929, 2864, 1640, 1548, 1455, 1373, 1252, 1195, 1116, 747, 699, 497 cm^{-1}.

2-Methyl-N-(2'-phenylethyl)butyramide (**1f**)

^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.26 (5H, m, Ph-H), 5.48 (1H, br s, N-H), 3.54 (2H, q, ^3J = 8.0 Hz, 1′-H_2), 2.82 (2H, t, ^3J = 8.0 Hz, 2′-H_2), 2.01 (1H, sext, ^3J = 8.0 Hz, 2-H), 1.63 (1H, h, ^3J = 8.0, 3-H), 1.39 (1H, h, ^3J = 8.0 Hz, 3-H), 1.07 (3H, d, ^3J = 8.0 Hz, 5-Me), 0.86 (3H, t, ^3J = 8.0 Hz, 4-Me). – ^{13}C NMR (acetone-*d*$_6$, 50.3 MHz) δ 176.3 (C_q-1), 140.6 (C_q-1"), 129.5 (CH-3", CH-5"), 129.1 (CH-2", CH-6"), 126.8 (CH-4"), 43.1 (CH_2-1′), 41.2 (CH-2), 36.6 (CH_2-2′), 27.9 (CH_2-3), 18.1 (CH_3-5), 12.2 (CH_3-4). – EI-MS (70 eV) *m/z* (%) 205 (M, 80), 104 (Ph-CH_2CH_2, 100), 85 (60), 57 (66). – IR (KBr) ν_{max} (cm^{-1}) 3316, 3086, 2963, 2928, 2873, 1742, 1641, 1557, 1453, 1380, 1233, 1029, 750, 701, 570, 492.

cyclo(Isoleucyldehydroalanyl) (**2**)

White solid, m.p. 215-220 °C (with decomposition), R_f = 0.20 ($CHCl_3$/5 % MeOH). – ^{1}H NMR (DMSO-*d*$_6$, 300 MHz) δ 10.44 (1H, br s, exchangeable with D_2O, 1-NH), 8.38 (1H, br s, exchangeable with D_2O, 4-NH), 5.18 (1H, s, 11-H_A), 4.78 (1H, s, 11-H_B), 3.96 (1H, dt, ^3J = 4.0 Hz, ^3J = 8.0 Hz, 6-H), 1.80 (1H, m, 8-H), 1.56 (2H, dt, ^4J = 2.0 Hz, ^3J = 8.0 Hz, 7-H_2), 0.86 (6H, d, ^3J = 8.0 Hz, 9, 10-CH_3). – ^{13}C NMR (DMSO-*d*$_6$, 50.3 MHz) δ 166.4 (C_q-5), 158.2 (C_q-2), 134.6 (C_q-3), 98.9 (CH_2-11), 53.7 (CH-6), 43.5 (CH_2-7), 22.6 (CH-8), 22.1 (CH_3-9, CH_3-10).– HMQC (DMSO-*d*$_6$, INVBTP, F1 125.7 MHz, F2 500 MHz) (H → C) 11-H → C-11; 11-H → C-11; 6-H → C-6; 8-H → C-8; 7-H → C-7; 9-H → C-9; 10-H → C-10. – HMBC (DMSO-*d*$_6$, IN4LPLRND, F1 125.7 MHz, F2 500 MHz) (H → C) 1-H ^2J → C-2; 1-H ^3J → C-7; 4-H ^2J → C-3, C-5; 11-H_2 ^2J → C-3; 11-H_2 ^3J → C-2; 11-H_2 ^4J → C-5; 6-H ^2J → C-5, C-7; 6-H ^3J → C-2, C-8; 8-H ^2J → C-7, C-9, C-10; 8-H ^3J → C-6; 7-H_2 ^3J → C-5; 9-H ^2J → C-8; 9-H ^3J → C-7; 10-H ^2J → C-8; 10-H ^3J → C-7. – DCI-MS (NH_3) *m/z* (%) 365 ($[2 M+1]^+$, 2), 217 ($[M+18+17]^+$, 24), 200 ($[M+18]^+$, 100), 183 ($[M+1]^+$, 12).

EI-HRMS Calcd for $C_9H_{14}N_2O_2$: 182.10552
Found: 182.1055

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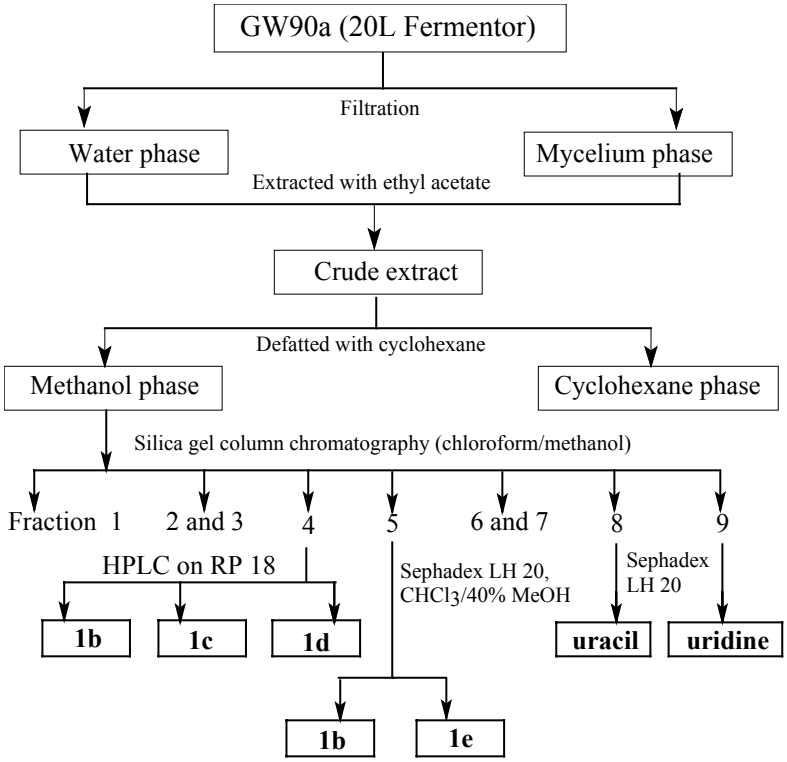
((Figure 1))



Work-up of the strain GW90a

((Figure 2))

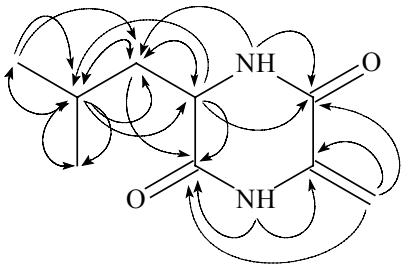


Figure 2. H,H-COSY (↔) and HMBC (→) couplings of *cyclo*(isoleucyldehydroalanyl) (**2**)

((Table 1))

Table 1. Physico-chemical Properties of Arylethylamides **1a-b**, **3**, and **4a-b**

	1a	**1b**	**1c**	**1d**	**1e**	**1f**	**3**	**4a**	**4b**
m.p. (°C)	85	52	80-81	64	57-58	57-58	118	70	120
R_f [a)]	0.48	0.56	0.63	0.65	0.65	0.65	0.78	0.61	0.78
M.F.	$C_{10}H_{13}NO$	$C_{11}H_{15}NO$	$C_{12}H_{17}NO$	$C_{13}H_{19}NO$	$C_{14}H_{21}NO$	$C_{13}H_{19}NO$	$C_{15}H_{15}NO$	$C_{10}H_{13}NO$	$C_{15}H_{15}NO$
EI-HRMS Calcd.	163.09971	177.11536	191.13101	205.14666	219.16231	205.14666	225.11536	163.09971	225.11536
Found	163.0997	177.1154	191.1310	205.1467	219.1623	205.1467	225.1154	163.0997	225.1154

[a)] eluent: $CHCl_3$/10 % MeOH

a)

((Table 2))

Table 2. Antimicroalgal activity of compounds **1b-1f**, and **3**, **4a** and **4b** in an agar plate diffusion assays at concentrations of 200 μg/disc.

	Diameter of Inhibition Zones (mm)		
	CV[b]	CS[c]	SS[d]
CE[a]	30	30	40
1a	0	0	0
1b	0	0	0
1c	0	0	0
1d	0	0	0
1e	16	13	20
1f	0	0	0
3	12	13	18
4a	0	0	0
4b	20	19	18

[a] Crude extract of GW90a, [b]*Chlorella vulgaris*,
[c] *Chlorella sorokiniana*, [d]*Scenedesmus subspicatus*

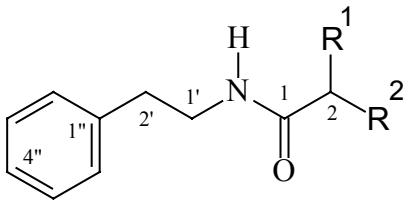
((Table 3))

Table 3. Antimicroalgal activity of phenylethylamides **1e, 3** and **4b** by serial dilution method; MIC (μg/ml)

	AD[a]	**1e**	**3**	**4b**
CV[b]	100	100	12.5	50
CS[c]	100	50	12.5	50
SS[d]	100	50	25	50

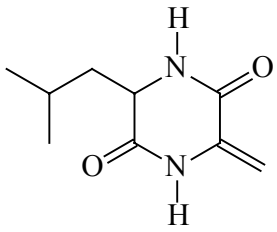
[a] Actinomycin C_2, [b]*Chlorella vulgaris*, [c]*Chlorella sorokiniana*, [d]*Scenedesmus subspicatus*

((Formula 1))



1	**a**	**b**	**c**	**d**	**e**	**f**
R^1	H	H	Me	H	H	Me
R^2	H	Me	Me	*i*-Pr	*n*-Bu	Et

((Formula 2-4))



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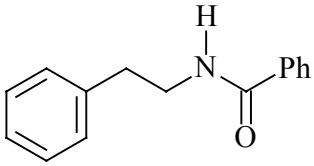

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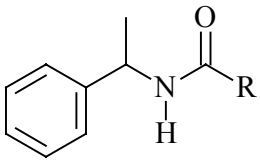
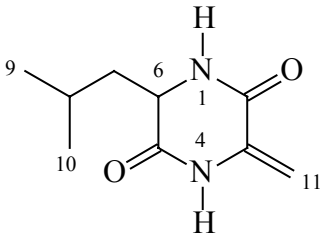


4a: R = Me; **4b**: R = Phe

**** or ****



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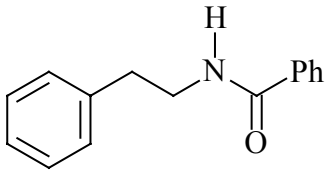

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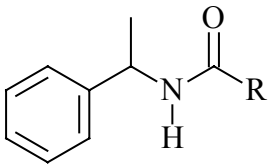

4a: R = Me; **4b**: R = Phe