Quinazolin-4-one derivatives from Streptomyces isolates

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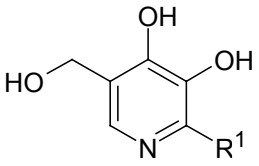
From the ethyl acetate extract of the strain *Streptomyces* sp. isolate GW23/1540, besides 16 known products, several 1*H*-quinazolin-4-one derivatives were isolated. (S*R*)-2-(1-Hydroxyethyl)-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**4**) and (R*R*)-2-(1-hydroxyethyl)-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**5**) are new natural products. 2-Methyl-3*H*-quinazolin-4-one (**2**) and 1*H*-quinazoline-2,4-dione (**3**) are known from other bacteria and plants, respectively. From another *Streptomyces* sp. GW2/577, 5-methyl-1*H*-quinazoline-2,4-dione (**6**) was isolated and the structure proven by comparison with the isomeric **7**. The new natural products showed no activity against the microalgae *Chlorella vulgaris, Chlorella sorokiniana,* and *Scenedesmus subspicatus,* the fungus *Mucor miehei*, the yeast *Candida albicans*, and the bacteria *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Streptomyces viridochromogenes*.

The quinazolin-4-one substructure has been found in many plant and microbial metabolites as part of complex molecules like tryptoquivaline,[1] circumdatin F and G,[2] tryptanthrine,[3] dipegine and dipeginol,[4] vasicinone and deoxyvasicinone.[5] Also some simple quinazoline derivatives have been reported, such as 2-benzyl-3*H*-quinazolin-4-one[6] or 1*H*-quinazoline-2,4-dione (**3**)[7] from plant sources, 2-acetyl-3*H*-quinazolin-4-one and 2-(1-hydroxyethyl)-3*H*-quinazolin-4-one from fungi.[8] From bacteria, however, only 2-methyl-3*H*-quinazolin-4-one, an inhibitor of the poly(ADP-ribose) synthetase, and 2,2-dimethyl-2,3-dihydro-1*H*-quinazolin-4-one have been isolated.[9] The related 2-benzyl-3*H*-quinazolin-4-one is active as a specific inhibitor of serine protease and human leucocyte elastase,[6] and 1,3-dimethyl-1*H*-quinazoline-2,4-dione shows anti-inflammatory, analgetic and anticonvulsant properties.[10] The inhibition of many other enzymes such as dihydrofolate reductase or caspase-3, have been reported.[11]

Results and Discussion

From the ethyl acetate extract of *Streptomyces* sp. isolate GW23/1540, we have now isolated (S*R*)-2-(1-hydroxyethyl)-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**4**) and (R*R*)-2-(1-hydroxyethyl)-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**5**) as new quinazolin-4-one derivatives. Additionally we have found the known 2-methyl-3*H*-quinazolin-4-one (**2**)[12] and as a secondary metabolite from microorganisms for the first time, 1*H*-quinazoline-2,4-dione (**3**).[7] Under similar conditions, the terrestrial streptomycete isolate GW2/577 produced 5-methyl-1*H*-quinazoline-2,4-dione (**6**). All these compounds were isolated as trace components in the usual yield of less than 1 mg/L culture broth.



1	**a**	**b**	**c**	**d**
R^1	*iso*-Pr	*n*-Pr	*sec*-Bu	*iso*-Bu

The strain GW23/1540 produced additionally the compounds **1a-d**, another group of new natural products with some similarity to vitamin B_6. They will be discussed in detail elsewhere. Further constituents isolated from *Streptomyces* sp. GW23/1540 were isobutyramide,[13,14] 2-methylbutyramide,[15] 3-indolylacetamide (so far known only from plants and sponges),[16] 3-(4-hydroxy-3-methoxyphenyl)acrylic acid,[17,18] 4-

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Dedicated to Prof. Dr. A. Zeeck on the occasion of his 65th birthday
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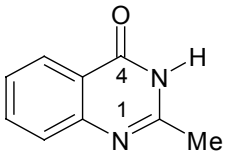
hydroxy-3-methoxybenzoic acid (vanillic acid, known from fungi and plants only),[19] and 3-(1-carboxyvinyloxy)benzoic acid[20] for the first time from bacteria. Besides these, the strain also produced 2-(*p*-hydroxyphenyl)ethylacetamide,[21] phenylacetamide,[22] pyrrole-2-carboxylic acid,[23] maltol,[24] 2-(3-indolyl)ethylacetamide,[25] 2-(3-indolyl)ethanol,[26] and 2-(*p*-hydroxyphenyl)ethanol.[27]

Compounds **2**-**5** were obtained from the low molecular weight fractions from Sephadex as colorless solids. They gave light blue fluorescence on TLC at 366 nm, and yellow spots on spraying with anisaldehyde/sulfuric acid, which turned to blue-violet and then yellowish after heating. The identical behavior pointed to similar structures.

The ESI mass spectrum afforded a molecular weight of 160 for compound **2**. A search with the NMR data and the molecular formula in AntiBase[28] afforded 2-methyl-3*H*-quinazolin-4-one (**2**). The structure was confirmed by comparison with reference data.[8,12]



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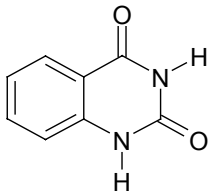
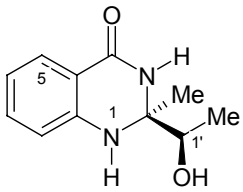


3

Compound **3** gave a ^{1}H NMR spectrum with the same pattern of downfield signals as that of **2**, however, the methyl singlet was absent here. A search with the molecular weight of 162 and the NMR data in the Dictionary of Natural Products[29] resulted in **3** as a possible structure. This was confirmed by comparison of the NMR data with the literature.[30]



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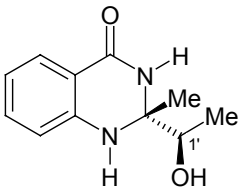
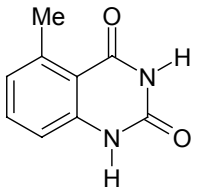


5

configuration at C-1' may be opposite

Though the ^{1}H NMR spectra of compounds **4** and **5** were identical in the region of aromatic and NH protons with those of **2** and **3**, they showed pronounced differences in the aliphatic part of the spectrum. They exhibited an OH signal at δ 4.4, a methine quartet at δ 3.9, a methyl singlet and a doublet at δ 1.4 and 1.2, respectively. The ^{13}C NMR spectrum of each compound showed a carbonyl signal at δ 164, six aromatic carbon signals between δ 148-114, a quaternary and a methine carbon connected to oxygen between δ 71-73 and two methyl carbons at about δ 22 and 17. The (+)-ESI mass spectra of both compounds gave *quasi*-molecular peaks at *m/z* 229 ($[M+Na]^+$) and 435 ($[2M+Na]^+$). The ESI HR mass spectrum led to the molecular formula $C_{11}H_{14}N_2O_2$ for both. The closely related NMR data and the identical molecular formula of compounds **4** and **5** indicated a diastereomeric relationship. The structures were confirmed by a detailed interpretation of the NMR data. If **4**/**5** are formed biosynthetically from the known bacterial products anthranilamide and diacetyl by cyclisation and further enzymatic reduction at C-1', it can be assumed that both compounds have identical configuration at C-1' and may both be either (*R*) or (*S*) at C-2'. Attempts to determine the chirality by means of Mosher derivatives were, however, unsuccessful. The cyclic aminals **4** and **5** are new natural products and related to the previously reported 2,2-dimethyl-2,3-dihydro-1*H*-quinazolin-4-one.[9]



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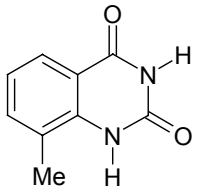
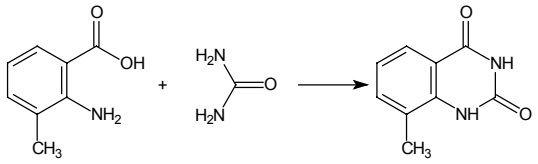


7

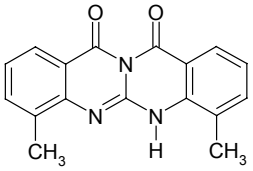
The molecular ion at *m/z* 176 in the EI-HRMS indicated the molecular formula $C_9H_8N_2O_2$ for **6**. The ^{1}H NMR spectrum of the compound showed three aromatic signals representing a 1,2,3-trisubtituted aromatic system. In addition, a 3H singlet could be assigned to an aromatic methyl group. The ^{13}C NMR spectrum contained nine signals as expected from the molecular formula. Comparison of the ^{13}C NMR data with those of **3** indicated it to be a methyl derivative of the latter. The two signals at δ 161.5 and 152.1 could be assigned to two carbonyl carbons, the residual sp^2 carbon signals to the aromatic ring and the signal at δ 21.5 to an aromatic methyl group. From the spectroscopic data and the molecular formula structures **6** and **7** were tentatively assigned, however, the available amount was insufficient to make a decision *via* HMBC spectra.

Both **6**[31] and **7**[11] have been obtained by synthesis previously, however, NMR data were not accessible. Compound **7** was thus synthesized from 3-methylanthranilic acid (**8**) and urea,[32] and the structure was proven by 2D NMR correlations. The NMR data of the natural product and **7** were very similar but not identical leading to structure **6** for the former. Compound **6** represents a new natural product, and also the synthetic isomer **7** has not been found in nature. It should be mentioned also that neither 3-, nor 6-methylanthranilic acid have been isolated from microorganisms so far.

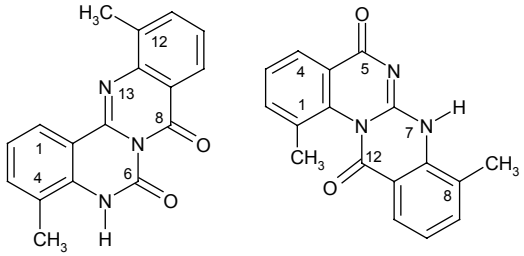


8 **7**

During the synthesis of **7**, a second minor reaction product was obtained. High resolution EIMS indicated a molecular ion at *m/z* 291, and hence the molecular formula $C_{17}H_{13}N_3O_2$. This indicated that **7** had reacted with a second molecule of 3-methylanthranilic acid. Three different isomers **9**-**11** can be expected, and all of them could exist as various prototropisomers.



9



10 **11**

Protonation of the azomethine nitrogen in **9** should result in a symmetrical ion. As addition of TFA did not enhance the symmetry of the ^{1}H NMR spectrum, **9** was excluded.

Only one signal (δ 159) in the ^{13}C NMR spectrum of the side product is in the expected range for an amide carbonyl and shows also an HMBC coupling with a *peri* proton (δ 7.96). The other candidates at δ 146.5 and 146.1 do not show long-range couplings (Figure 1) and differ strongly from shifts in related compounds: The corresponding CO signals in **3**, **6** or **7** are in the range of δ 150-152, and also shift predictions using computer programs have not been helpful.[33] The $\Delta^{13,13a}$ azomethine bond in **10** should influence adjacent atoms weaker than a carbonyl group, as comparison with the shifts of the *peri* protons in **2** indicates. The strongly deshielded methyl signal (δ 2.63) and the presence of *two* downfield *peri*-protons (shifts as in **2**, **3** or **7**) cannot be explained readily by structure **10**, however, fit nicely with **11**. The structure and the mode of formation of **11** from **7** and **8** show some similarity with the yeast pigment tryptanthrine,[34] which can be assembled in a similar way from isatin and anthranilic acid.

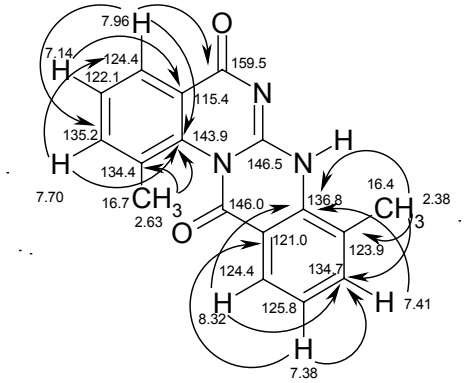


Figure 1: HMBC couplings of **11** in d_6-DMSO

The known metabolites isobutyramide,[13] 2-methylbutyramide,[15] 3-indolylacetamide,[16] 3-(4-hydroxy-3-methoxyphenyl)acrylic acid,[17] 4-hydroxy-3-methoxybenzoic acid (vanillic acid,[19] 3-(1-carboxyvinyloxy)benzoic acid,[20] 2-(*p*-hydroxyphenyl)ethylacetamide,[21] phenylacetamide,[22] pyrrole-2-carboxylic acid,[23] maltol,[24] 2-(3-indolyl)ethylacetamide,[25] 2-(3-indolyl)ethanol,[26] and 2-(*p*-hydroxyphenyl)ethanol[27] were also obtained from strain GW23/1540 and identified by NMR and MS data using AntiBase.[28]

Experimental Section

General Experimental Procedures. NMR spectra were measured on Mercury-200 (199.993 MHz), AMX 300 (300.135 MHz), Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometer. ESIMS spectra were recorded on a Quattro Triple Quadrupole Mass Spectrometer, Finnigan TSQ 7000 with nano-ESI-API ion source. EIMS spectra were recorded on Varian MAT 731 (70 eV), Varian 311A (70 eV), and AMD-402 (70 eV).

Reserpine and leucin-enkephalin were used as reference substances in (+)- and (-)-ESI-HRMS. IR spectra were recorded on a Perkin-Elmer 1600 Series FT-IR spectrometer as KBr pellets. Preparative HPLC was performed using an RP18 column (Eurochrom Eurospher RP 100-C18, 5 µm) using a Knauer variable wavelength monitor at 202 nm. Flash chromatography was carried out on silica gel (230-400 mesh). Thin layer chromatography (TLC) were performed on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.), R_f values were measured with 10 % MeOH in CH_2Cl_2 when not stated otherwise. Size exclusion chromatography was done on Sephadex LH-20 (Pharmacia).

Malt extract/yeast extract/glucose-medium: Malt extract (10 g), yeast extract (4 g) and glucose (4 g) were dissolved in 1 L of tap water and the medium was adjusted to pH 7.8 with 2 N NaOH and sterilized for 33 min at 121 °C. After sterilization, an end pH 7.0 of the medium was attained.

Actinomycete GW 2/577: The strain was obtained from the strain collection of the Labor für Bodenmikrobiologie, Burggarten 9, D-35102 Lohra-Kirchvers, Germany. It was gram-positive, non-acid fast, grew aerobically, and differentiated into substrate and aerial mycelium.

The aerial mycelium displayed short and branched *flexuous* spore chains. Neither aerial hyphae nor substrate mycelium showed fragmentation. Other morphological features such as sporangia, sclerotia, synnemata or motile spores, were not observed.

The color of the aerial mycelium was cream white with shades of pink on yeast extract-malt agar, and cream on oatmeal agar. The substrate mycelium was red brown on most media. A brown diffusible pigment was formed on all media. Melanin pigments were produced on tyrosine agar.

***Streptomyces* sp. GW23/1540**: The actinomycete isolate GW23/1540 was obtained from the strain collection of the Labor für Bodenmikrobiologie (see above). Cells of this strain were aerobic, gram-positive, non-acid fast, and filamentous. The strain formed an extensive substrate mycelium and aerial hyphae with *spiral* chains of spores and a pseudo-verticillate arrangement of sporophores. The color of the aerial mycelium was gray and pale red, and the reverse was brown. The strain did not produce melanoid pigments on tyrosine agar or peptone iron agar.

Based on chemotaxonomic properties like presence of L,L-diaminopimelic acid, absence of characteristic sugars, growth, and morphology the strain GW 23/1540 most probably belongs to the genus *Streptomyces*.

Fermentation of strain GW23/1540, extraction and separation: 180 of 1 L Erlenmeyer flasks each containing 250 mL malt extract/yeast extract/glucose medium were inoculated with the well grown agar plates of the producing strain *Streptomyces* sp. GW23/1540 and grown for 72 h at 28 °C while shaking at 95 rpm (circular shaker ITE, Infors, Germany). The combined culture broth was mixed with celite (ca. 1.5 kg) and filtered through a press filter. The culture filtrate and biomass were each extracted separately with EtOAc. Since the metabolic spectra were similar in both organic phases, they were combined and evaporated to dryness to yield 8.2 g of crude extract.

The crude extract obtained from the strain GW23/1540 was dissolved in 150 mL of MeOH and defatted by extracting with 150 mL of cyclohexane. The MeOH phase gave 6.8 g of dry extract that was subjected to column chromatography on Sephadex LH-20 (CH_2Cl_2/70 % MeOH) to give three fractions.

Fraction 2 was chromatographed on silica gel (flash column 2 × 20 cm, 100 g) with a stepwise CH_2Cl_2/MeOH gradient (1.5 L $CHCl_3$, 1.5 L $CHCl_3$/2% MeOH, 1 L $CHCl_3$ /10% MeOH). Further purification of the five subfractions by preparative HPLC and then PTLC afforded (RR)-2-(1-hydroxyethyl)-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**4**, 5 mg, R_f = 0.28), (RS)-2-(1-hydroxyethyl)-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**5**, 26 mg, R_f = 0.28), 2-methyl-3H-quinazolin-4-one (**2**, 13 mg, R_f = 0.37), 1*H*-quinazoline-2,4-dione (**3**, 5 mg, R_f = 0.46), isobutyramide (223 mg, R_f = 0.42), 2-methylbutyramide (110 mg, R_f = 0.37), 3-indolylacetamide (25 mg, R_f = 0.35), 3-(4-hydroxy-3-methoxyphenyl)acrylic acid (8.3 mg, R_f = 0.27), 4-hydroxy-3-methoxy-benzoic acid (4.3 mg, R_f = 0.32), 3-([1-carboxyvinyl]oxy)-benzoic acid (2.5 mg, R_f = 0.21), 2-(*p*-hydroxyphenyl)ethylacetamide (30 mg, R_f = 0.37), phenylacetamide (20 mg, R_f = 0.40), pyrrole-2-carboxylic acid (25 mg, R_f = 0.40), maltol (2 mg, R_f = 0.38), 2-(3-indolyl)ethylacetamide (26 mg, R_f = 0.48), 2-(3-indolyl)ethanol (4 mg, R_f = 0.46), 2-(*p*-hydroxyphenyl)etha-

nol (21 mg, R_f = 0.42), and **1** (64 mg, R_f = 0.43 - 0.45).

Fermentation of the strain GW2/577, extraction and separation: A well grown 3 L shaker culture of isolate GW2/577 incubated at 28 °C and 95 rpm for 3 days in the same medium as above was used to inoculate a 30 L fermentor containing 24 L of the medium. The culture was grown for 72 h at a pH 6.5 ± 1.25 and worked up similar as before to deliver 10.1 g of crude extract.

After defatting, the crude extract (5.2 g) was subjected to a flash column chromatography on silica gel ($CHCl_3$ 1 L, $CHCl_3$ /1 % MeOH 0.5 L, $CHCl_3$ / 3% MeOH 0.5 L, $CHCl_3$ /5% MeOH 1 L, $CHCl_3$ 10 %MeOH 1 L, $CHCl_3$ /20% MeOH 0.2 L) to deliver four fractions.

After successive purification on Sephadex LH-20 ($CHCl_3$/40 % MeOH) and preparative HPLC (H_2O/CH_3CN), fraction 3 delivered 2 mg of 5-methyl-1*H*-quinazoline-2,4-dione (**6**).

(S*,R*)-2-(1-Hydroxyethyl)-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**4**): Colorless solid, R_f = 0.28; $[\alpha]^{20}_D$ +47.0° (c 0.2, MeOH); UV (MeOH) λ_{max} (lg ε) 252 (3.61), 348 (3.33); IR (KBr) ν_{max} 3310, 2973, 2948, 1650, 1637, 1619, 1515, 1487, 1384, 1334, 1274, 1154, 1105, 1009, 756, 702, 555 cm^{-1}; ^1H NMR (acetone-d_6, 300.2 MHz) δ 7.69 (dd, ^3J = 7.9 Hz, ^4J = 1.6 Hz, 1 H, H-5), 7.26 (br s, 1 H, NH), 7.21 (ddd, ^3J = 8.3, and 7.1 Hz, ^4J = 1.6 Hz, 1 H, H-7), 6.73 (dd, ^3J = 8.3 Hz, ^4J = 1.1 Hz, 1 H, H-6), 6.64 (ddd, ^3J = 7.9 and 7.1 Hz, ^4J = 1.1 Hz, 1 H, H-8), 5.99 (br s, 1 H, NH), 4.42 (br s, 1 H, OH), 3.91 (q, ^3J = 6.4 Hz, 1 H, H-1'), 1.46 (s, 3 H, 2-CH_3), 1.20 (d, ^3J = 6.4 Hz, 3 H, CH_3-2'); ^{13}C NMR (acetone-d_6, 75.5 MHz) δ 164.4 (CO), 147.8 (C-8a), 134.2 (CH-7), 128.2 (CH-5), 117.6 (CH-8), 115.3 (CH-6), 115.0 (C-4a), 72.7 (C-2), 71.8 (CH-1'), 22.7 (CH_3-2), 17.6 (CH_3-2'); EI-MS (70 eV) *m/z* (%) 338 $[M]^+$ (100), 221 (9), 208 (4), 169 (8), 154 (4), 130 (48), 103 (3), 83 (8), 77 (4), 57 (3); (+)-ESI-MS *m/z* (%) 641 $[3M+Na]^+$ (17), 435 $[2M+Na]^+$ (100), 229 $[M+Na]^+$ (8); ESI-HRMS 206.1034 (calcd. 206.105527 for $C_{11}H_{14}N_2O_2$), 229.0932 (calcd. 229.095297 for $C_{11}H_{14}N_2O_2Na$, $[M+Na]^+$).

(R*,R*)-2-(1-Hydroxyethyl)-2-methyl-2,3-dihydro-1*H*-quinazolin-4-one (**5**): Colorless solid, R_f = 0.28; $[\alpha]^{20}_D$ –17.5° (c 0.2, MeOH); UV (MeOH) λ_{max} (lg ε) 253 (3.51), 350 (3.25); IR (KBr) ν_{max} 3402, 2980, 2943, 1654, 1636, 1596, 1517, 1487, 1384, 1334, 1275, 1152, 1101, 1032, 911, 755, 702, 559 cm^{-1}; ^1H NMR (acetone-d_6, 300.2 MHz) δ 7.63 (dd, ^3J = 7.9 Hz, ^4J = 1.6 Hz, 1 H, H-5), 7.19 (br s, 1 H, NH), 7.19 (ddd, ^3J = 8.2 and 7.2 Hz, ^4J = 1.6 Hz, 1 H, H-7), 6.75 (dd, ^3J = 8.2 Hz, ^4J = 1.1 Hz, 1 H, H-8), 6.63 (ddd, ^3J = 7.9 and 7.2 Hz, ^4J = 1.1 Hz, 1 H, H-6), 5.87 (br, 1 H; NH), 4.43 (s br, 1 H, OH), 3.90 (q, ^3J = 6.4 Hz, 1 H, H-1'), 1.46 (s, 3 H, 2-CH_3), 1.20 (d, ^3J = 6.4 Hz, 3 H, H_3-2'); ^{13}C NMR (acetone-d_6, 75.5 MHz) δ 164.2 (CO), 148.0 (C-8a), 134.2 (CH-7), 128.2 (CH-5), 117.4 (CH-8), 114.8 (CH-6), 114.8 (C-4a), 72.9 (C-2), 72.9 (CH-1'), 23.5 (CH_3-2), 17.8 (CH_3-2'); ^{13}C NMR (CD_3OD, 75.5 MHz) δ 166.5 (CO), 148.7 (C-8a), 135.3 (CH-7), 128.4 (CH-5), 118.0 (CH-8), 115.1 (CH-6), 114.3 (C-4a), 73.3 (CH-1'), 73.1 (C-2), 23.0 (CH_3-2), 17.5 (CH_3-2'); EI-MS (70 eV) *m/z* (%) 161 $[M-CHOHCH_3]^+$ (100), 221 (9), 208 (4), 169 (8), 154 (4), 130 (48), 103 (3), 83 (8), 77 (4), 57 (3); (+)-ESI-MS *m/z* (%) 641 $[3M+Na]^+$ (8), 435 $[2M+Na]^+$ (100), 229 $[M+Na]^+$ (10); ESI-HRMS 206.1034 (calcd. 206.10553 for $C_{11}H_{14}N_2O_2$), 229.0932 (calcd. 229.09530 for $C_{11}H_{14}N_2O_2Na$, $[M+Na]^+$).

5-Methyl-1*H*-quinazoline-2,4-dione (6): R_f = 0.50 ($CHCl_3$/ 10 % MeOH), UV absorbing at 254 nm, no color reaction with anisaldehyde/ sulfuric acid or Ehrlich's reagent. ^1H NMR (acetone-d_6, 300.2 MHz) δ 8.30 (s br, 1 H; not in publ.), 7.57 (dd, ^3J = 7.9 Hz, ^4J = 1.5 Hz, 1 H, H-8), 7.30 (t, ^3J = 7.9 Hz, 1 H, H-7), 7.19 (dd, ^3J = 7.9 Hz, ^4J = 1.5 Hz, 1 H, H-6), 2.48 (s, 3 H, Me-5); ^{13}C NMR (acetone-d_6, 50.3 MHz) δ 161.5 (C-4), 152.1 (C-2), 139.7 (C-8a), 137.7 (C-5), 126.7 (CH-7), 121.5 (C-4a), 117.2 (CH-6), 116.1 (CH-8), 21.1 (CH_3-5); EI-MS (70 eV) *m/z* (%) 176 $[M]^+$ (100), 162 $[M-CH_3]^+$ (21), 151 (30), 134 [M - O=C=N]$^+$ (22), 121 (13), 107 (24), 91 (16.5), 65 (10), 51 (11); DCI-MS (NH_3) *m/z* (%) 353 $[2M+H]^+$ (4), 194 $([M+NH_4]^+$ (28), 177 $[M+H]^+$ (100); ESI-HRMS 176.0590 (calcd 176.05857 for $C_9H_8N_2O_2$).

8-Methyl-1*H*-quinazoline-2,4-dione (7): A mixture of 4 mmol (600 mg) of 3-methylanthranilic acid and 8 mmol (480 mg) urea was molten together and kept for 5 min at about 125 °C. After cooling to room temperature, the resulting solid was triturated with 200

mL of $CHCl_3$/MeOH (87:13) and the undissolved material discarded. Separation of the crude product by PTLC (20 × 20 cm, $CHCl_3$/10 % MeOH) and Sephadex LH-20 ($CHCl_3$/MeOH 6:4) afforded 500 mg (71 %) of **7** as colorless needles after crystallization from MeOH. Purification of the mother liquor gave 10 mg of **11** as a yellow byproduct.

7: R_f = 0.52 ($CHCl_3$/ 10% MeOH, mp 297-299 °C; IR (KBr) ν_{max} 3508, 3428, 3025, 2845, 1721, 1683, 1635, 1507, 1459, 1418, 1326, 1085, 882, 752, 532 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300.2 MHz) δ 10.80 (br s, 2 H, 2 NH), 7.88 (dd, ^3J = 7.6 Hz, ^4J = 1.1 Hz, 1 H, H-5), 7.54 (dd, ^3J = 7.6 Hz, ^4J = 1 Hz, 1 H, H-7), 7.09 (t, ^3J = 7.6 Hz, 1 H, H-6), 2.36 (s, 3 H, CH_3-8); ^{1}H NMR (acetone-d_6, 300.2 MHz) δ 10.10 (br s, 1 H, NH), 9.25 (br s, 1 H, NH), 7.88 (dd, ^3J = 7.6 Hz, ^4J = 1.1 Hz, 1 H, H-5), 7.54 (dd, ^3J = 7.6 Hz, ^4J = 1 Hz, 1 H, H-7), 7.12 (t, ^3J = 7.6 Hz, 1 H, H-6), 2.47 (s, 3 H, CH_3-8); ^{13}C NMR (DMSO-d_6, 50.3 MHz) δ 162.9 (CO-4), 150.4 (CO-2), 139.1 (C-8a), 135.9 (CH-7), 124.7 (CH-5), 124.0 (C-8), 122.0 (CH-6), 114.6 (C-4a), 17.0 (CH_3-5); EI MS (70 eV) *m/z* (%) 176 $[M]^+$ (100), 133 [M - C=O-NH]$^+$ (28), 105 [M - (C=O-NH-C=O)]$^+$ (63), 77.0 (14), 51.0 (8); *anal*. C 61.64 %, H 4.83 %, calcd. for $C_9H_8N_2O_2$, C 61.36 %, H 4.58 %.

4,12-Dimethyl-5*H*-quinazolino[4,3-b]quinazoline-6,8-dione (11): R_f = 0.52 ($CHCl_3$/ 10% MeOH; m.p. 288-290 °C. ^{1}H NMR (DMSO-d_6, 599.878 MHz) δ 8.32 (dd, ^3J = 7.6 Hz, ^4J = 1.1 Hz, 1 H, H-11), 7.96 (dd, ^3J = 7.6 Hz, ^4J = 1 Hz, 1 H, H-4), 7.7 (dd, ^3J = 7.6, ^4J = 1 Hz, 1 H, H-2), 7.4 (dd, ^3J = 7.6, ^4J = 1 Hz, 1 H, H-9), 7.38 (t, ^3J = 7.6 Hz, 1 H, H-10), 7.14 (t, ^3J = 7.6 Hz, 1 H, H-3), 2.63 (s, 3 H, CH_3-1), 2.38 (s, 3 H, CH_3-8); ^{13}C NMR/APT (DMSO-d_6, 125.707 MHz) δ 159.47 (C_q-5), 146.49 (C_q-6), 146.06 (C_q-12), 143.9 (C_q-1a), 136.81 (C_q-7a), 135.23 (CH-2), 134.7 (CH-9), 134.40 (C_q-1), 125.83 (CH-10), 124.4 (CH-11), 124.4 (CH-4), 123.9 (C_q-8), 122.12 (CH-3), 121.02 (C_q-11a), 115.41 (C_q-4a), 16.69 (CH_3-1), 16.46 (CH_3-8); EI MS (70 eV) *m/z* (%) 291.2 $[M]^+$ (100), 274.1 $[M - NH_3]^+$ (30), 262.2 [M - (HC=O)]$^+$ (63), (12), 159.1 (7), 145.6 (6), 105.1 (8), 77.0 (5); EIHRS found 291.1008, calcd. 291.1007 for $C_{17}H_{13}N_3O_2$).

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