Novel 3-(2-Oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoates from Dimethyl-2-oxoglutarate and Test of Their Biological Activity

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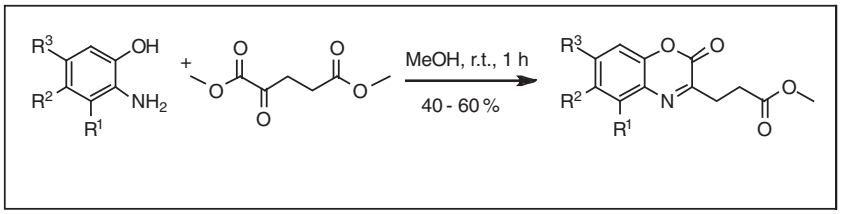

Five novel 3-(2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoates were synthesized under mild conditions from 2-aminophenols and dimethyl-2-oxoglutarate. Biological assays of these 1,4-benzoxazinones were conducted with three bacterial strains and one yeast. All compounds were active against a *Candida albicans* ATCC 10231, whereas only methyl 3-(6-methyl-2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoate showed a general moderate activity against the bacterial strains tested.

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INTRODUCTION

This article was inspired by two conceptually different areas of our research. On the one hand, 2-oxoglutaric acid, well known as a part of the citric acid cycle, is—in accordance to the *White biotechnology for green chemistry* concept—a 2-oxocarboxylic acid [1] now accessible in large quantities by fermentation of paraffines, glycerol, ethanol, glucose, or sunflower oil. Hence, 2-oxoglutaric acid is on its way from a laboratory chemical to a biotechnologically manufactured industrial product [2–4]. Earlier, we reported on the synthesis of heterocycles based on this building block [5]. On the other hand, naturally occurring 2*H*-1,4-benzoxazin-3 (4*H*)-ones from cereal plants are well known for their high biological activity as intrinsic resistance factors of these plants [6]. From this perspective, 1,4-benzoxazines are of general interest for biological testing. The versatility of related 1, 4-benzoxazin-2-ones is similarly linked to their potential pharmacological uses [7–9] and their photochemical activity [10–13]. For example, 1,4-benzoxazin-2-ones prepared from *o*-aminophenols and methyl acylpyruvates show significant antimicrobial activities against *Staphylococcus aureus* and *Escherichia coli*. Common strategies that have been employed for their synthesis are the reaction of substituted 2-aminophenols with 2-oxoesters [14–17], alkyl propiolates [18], or β-nitroacrylates [19].

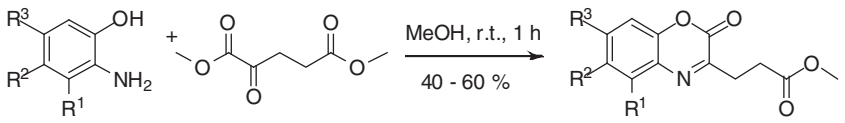
Here, we report a facile synthesis of novel 3-(2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoates from 2-aminophenols and dimethyl-2-oxoglutarate and results of their antimicrobial and antifungal testing.

RESULTS AND DISCUSSION

Synthesis of 3-(2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoates 3a–e. Dimethyl-2-oxoglutarate **2** (Scheme 1) was prepared in a high yield (85%) by esterification of 2-oxoglutaric acid with methanol according to the procedure formerly reported [20]. As shown in Scheme 1, the reaction of dimethyl-2-oxoglutarate with an equimolar amount of 2-aminophenol **1a**, 2-amino-5-methylphenol **1b**, 2-amino-4-methylphenol **1c**, 2-amino-3-methylphenol **1d**, and 2-amino-4-chlorophenol **1e** afforded the corresponding 3-(2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoates **3a–e** in good yields under very mild conditions. All novel compounds **3a–e** obtained have been characterized by spectroscopic data and elemental analysis as shown in the Experimental section. The commercial availability of 2-aminophenols **1a–e** was the reason for the pattern of substituents described here. Heterocycles **3a–e** have not been reported yet.

Antimicrobial activity of compounds 3a–e. Biological activity of the products (**3a–e**) in form of DMSO solutions was assessed against fungus and yeast by the method of discs, also known as Bauer-Kirby method as described in the Biological Assay section. The biological strains studied were *S. aureus* ATCC 6538 (gram-positive bacteria), *E. coli* ATCC 10536 and *Proteus vulgaris* ATCC 6896 (gram-negative bacteria), and *Candida albicans* ATCC 10231 (yeast). A blank test was performed with DMSO, and the result was negative, that is, no aureola was formed indicating no inhibition of microbial growth. The

Scheme 1. Synthetic route to substituted 3-(2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoates (**3a–e**).



1 a -e **2** **3 a - e**

1,3	R^1	R^2	R^3
a	H	H	H
b	H	H	CH_3
c	H	CH_3	H
d	CH_3	H	H
e	H	Cl	H

screening results shown in Table 1 clearly suggest that all compounds exhibited antifungal activity in the order **3a** > **3c** > **3d** > **3e** > **3b**. Conspicuously, methyl 3-(6-methyl-2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoate (**3c**) was active towards all the biological strains, and its antifungal activity was 2.5 times higher than its antibacterial one. Compounds **3a** and **3e** were active against *P. vulgaris* ATCC 6896, and compound **3c** was active against all bacteria strains tested. No other suppression of bacterial growth was observed. On the other hand, the minimum inhibiting concentrations (MIC), defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after incubation, of 1,4-benzoxazin-2-ones were also determined [21] (Table 1). As shown, all MICs were found to be in the spectrum of 1 250 to 2 500 μg/mL for **3c** towards *S. aureus* ATCC 6538, *E. coli* ATCC 10536 and *P. vulgaris* ATCC 6896. It is worth recalling that the reported MICs for (7)-substitued-3-acylmethylene-1,4-benzoxazin-2-ones and ethacridine lactate towards the first two bacteria were in the range of 500–2000 μg/mL [22]. The antimicrobial activity of the present compounds strongly depends on the type of strain tested and the nature and position of the substituent at the benzene ring. Clearly, the 6-methyl derivative **3c** showed the highest bioactivity.

In summary, five novel 3-(2-oxo-2*H*-benzo[*b*][1,4] oxazin-3-yl)propanoates (**3a–e)** have been prepared from dimethyl-2-oxoglutarate, and their biological activity has been tested toward three bacterial strains and one yeast. All compounds were active against a *C. albicans* strain, whereas only **3c** clearly showed the highest activity against the bacteria strains tested.

A statement such as this is a typical feature of experiments with substituents of different kind and at different positions, for example, at aromatic rings. The reasons are very difficult to unravel in a preliminary investigation as described here. However, it is known from many examples that already minor changes may alter the biological performance of small molecules dramatically. The difference in the behavior of the allelopathic natural products benzoxazolin-2(3*H*)-one and 6-methoxybenzoxazolin-2(3*H*)-one known from rye and maize exudates, respectively, is one of the most convincing examples [23].

Table 1

Results of the antimicrobial tests of **3a–e**; concentration 160 mg/mL in DMSO.

	Antibacterial activity[a]			
	Gram-positive (+)	Gram-negative (−)		Antifungal activity[a]
Compound tested	*Staphylococcus aureus* ATCC 6538	*Escherichia coli* ATCC 10536	*Proteus vulgaris* ATCC 6896	*Candida albicans* ATCC 10231
3a	00	00	09 (σ = 0.47)(20 000)	34 (σ = 0.94)(10 000)
3b	00	00	00	16 (σ = 0.47)(5000)
3c	13 (σ = 2.49)(2500)	13 (σ = 2.16)(2500)	12 (σ = 0.47)(1250)	30 (σ = 0.94)(40 000)
3d	00	00	00	25 (σ = 0.47)(20 000)
3e	00	00	13 (σ = 0.47)(40 000)	21 (σ = 1.41)(10 000)

[a]Diameter of aureola formed in millimeter (mm); the mean of three experimental values, and σ is the standard deviation; the numbers below and in parentheses are the minimum inhibiting concentration values in μg/mL. The minimum inhibitory concentration was assessed as described in the literature using the method of dilution [21].

EXPERIMENTAL

Melting points were determined on a Boetius micro hot stage and are corrected. IR spectra were measured in KBr on spectrophotometer Specord M80, Carl Zeiss, Jena, Germany.. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 with Varian Mercury plus 300 or 400 MHz spectrometers. Chemical shifts for NMR signals are reported in parts per million from tetramethylsilane. 70 eV Electron impact mass spectra were recorded with a MAT 8230 Thermo Finnigan mass spectrometer. Elemental analyses were measured with a Vario El elemental analyzer from Elementar Analysensysteme GmbH Hanau, Germany.

Dimethyl-2-oxoglutarate (2). A 73.05 g (0.5 mole) of 2-oxoglutaric acid was dissolved in dry methanol (300 mL) and allowed to stand for 5 days at 20°C. A 104.5 g (1.0 mole) of 2,2-dimethoxypropane was added, and the solution was allowed to stand for an additional day. Methanol and acetone were removed in vacuo. The remaining oily crude product was then subjected to a fractional distillation in vacuo. Dimethyl-2-oxoglutarate was isolated as colorless oil, 74.0 g (85%), bp 102–104°C/ 2.66 mbar, n_D^{20} 1.4414.

General procedure for the synthesis of substituted 3-(2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoates 3a–e. A solution of 1.74 g (0.01 mole) of dimethyl-2-oxoglutarate (**2**) in 10 mL of dry methanol was added dropwise under stirring to a solution of the corresponding substituted 2-aminophenol (**1a–e**) (0.01 mole) in 20 mL of dry methanol at 20°C. After stirring for 1 h at 20°C, the crystals formed were filtered off and recrystallized from methanol.

Methyl 3-(2-oxo-2H-benzo[b][1,4]oxazin-3-yl)propanoate (3a). This compound was obtained as orange crystals, 1.39 g, (60%), mp 134–135°C; ir: ν CO 1740 cm^{-1} (C═O), N═C 1612 cm^{-1}; ^{1}H NMR: δ 2.76 (t, 2H, CH_2, J = 7.2 Hz); 3.03 (t, 2H, CH_2, J = 7.2 Hz); 3.59 (s, 3H, OCH_3); 7.36 (dd, 1H, H_{arom}, J = 8.6 Hz, J = 7.5 Hz); 7.38 (d, 1H, H_{arom}, J = 8.5 Hz); 7.51 (dd, 1H, H_{arom}, J = 8.5 Hz, 7.5 Hz); 7.67 (d, 1H, H_{arom}, J = 8.6 Hz). ^{13}C NMR: δ 28.14 (CH_2); 29.11 (CH_2); 51.48 (OCH_3); 116.23 (C-8); 125.31 (C-6); 128.24 (C-5); 130.49 (C-7); 130.57 (C-4a); 146.10 (C-8a); 152.28 (C═O); 156.60 (N═C); 172.67 (COO); ms: *m/z* 233 (M^+, 58%), 231 ($M^+ - OCH_3$, 37%), 174 ($M^+ - CO_2CH_3$, 35%), 146 ($M^+ - CH_2$-CH_2-CO_2CH_3, 100%). *Anal*. Calcd for $C_{12}H_{11}NO_4$: C, 61.80; H, 4.75; N, 6.01. Found: C, 61.63; H, 4.74; N, 5.91.

Methyl 3-(7-methyl-2-oxo-2H-benzo[b][1,4]oxazin-3-yl)propanoate (3b). This compound was obtained as colorless crystals, 0.98 g (40%), mp 99–100°C; ir ν C═O 1740, N═C 1616 cm^{-1}; ^{1}H NMR: δ 2.38 (s, 3H, CH_3); 2.74 (t, 2H, CH_2, J = 7.1 Hz); 3.01 (t, 2H, CH_2, J = 7.1 Hz); 3.59 (s, 3H, OCH_3); 7.18 (d, 1H, H_{arom}, J = 8.4 Hz); 7.20 (s, 1H, H_{arom}); 7.54 (d, 1H, H_{arom}, J = 8.4 Hz). ^{13}C NMR: δ 20.98 (CH_3); 28.02 (CH_2); 29.12 (CH_2); 51.44 (OCH_3); 116.18 (C-8); 126.20 (C-6); 127.85 (C-5); 128.55 (C-7); 141.17 (C-4a); 145.93 (C-8a); 152.69 (C═O); 155.21 (N═C); 172.66 (COO); ms: *m/z* 247 (M^+, 88%), 216 ($M^+ - OCH_3$, 51%), 188 ($M^+ - CO_2CH_3$, 36%), 160 ($M^+ - CH_2$-CH_2-CO_2CH_3, 100%). *Anal*. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.08; H, 5.11; N, 5.61.

Methyl 3-(6-methyl-2-oxo-2H-benzo[b][1,4]oxazin-3-yl)propanoate (3c). This compound was obtained as colorless crystals,1.23 g (50%), mp 100–101°C; ir ν C═O 1740, N═C 1612 cm^{-1}; ^{1}H NMR: δ 2.35 (s, 3H, CH_3); 2.74 (t, 2H, CH_2, J = 7.1 Hz, CH_2); 3.02 (t, 2H, CH_2, J = 7.1 Hz); 3.59 (3H, s, OCH_3); 7.25 (d, 1H, H_{arom}, J = 8.7 Hz); 7.34 (dd, 1H, H_{arom}, J = 8.7 Hz, J = 1.5 Hz); 7.46 (d, 1H, H_{arom}, J = 1.5 Hz). ^{13}C NMR: δ 20.16 (CH_3); 28.08 (CH_2); 29.06 (CH_2); 51.45 (OCH_3); 115.81 (C-8); 128.04 (C-6); 130.24 (C-5); 131.15 (C-7); 134.79 (C-4a); 143.97 (C-8a); 152.69 (C═O); 156.37 (N═C); 172.63 (COO); ms: *m/z* 247 (M^+, 81%), 216 ($M^+ - OCH_3$, 43%), 188 ($M^+ - CO_2CH_3$, 19%), 160 ($M^+ - CH_2$-CH_2-CO_2CH_3, 100%). *Anal*. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.03; H, 5.04; N, 5.59.

Methyl 3-(5-methyl-2-oxo-2H-benzo[b][1,4]oxazin-3-yl)propanoate (3d). This compound was obtained as colorless crystals, 1.23 g (50%), mp 132–134°C; ir ν C═O 1740, N═C, 1612 cm^{-1};- ^{1}H NMR: δ 2.48 (s, 3H, CH_3); 2.74 (t, 2H, CH_2, J = 6.6 Hz); 3.04 (t, 2H, CH_2, J = 6.6 Hz); 3.60 (s, 3H, OCH_3); 7.17 (d, 1H, H_{arom}, J = 8.4 Hz); 7.22 (d, 1H, $H_{arom.}$, J = 6.9 Hz) 7.39 (dd, 1H, H_{arom}, J = 8.4 Hz, J = 6.9 Hz). ^{13}C NMR: δ 16.11 (CH_3); 28.17 (CH_2); 29.05 (CH_2); 51.35 (OCH_3); 113.81 (C-8); 126.24 (C-6); 128.87 (C-5); 130.11 (C-7); 136.92 (C-4a); 146.28 (C-8a); 152.53 (C═O); 154.73 (N═C); 172.73 (COO); ms: *m/z* 247 (M^+, 80%), 215 ($M^+ - CH_3OH$, 100%), 188 ($M^+ - CO_2CH_3$, 10%), 160 ($M^+ - CH_2$-CH_2-CO_2CH_3, 80%). *Anal*. Calcd for $C_{13}H_{13}NO_4$: C, 63.15; H, 5.30; N, 5.67. Found: C, 63.10; H, 5.14; N, 5.58.

Methyl 3-(6-chloro-2-oxo-2H-benzo[b][1,4]oxazin-3-yl)propanoate (3e). This compound was obtained as colorless crystals, 1.23 g (50%), mp 114–116°C; ir ν C═O 1740, N═C 1615 cm^{-1} (N═C); ^{1}H NMR: δ 2.77 (t, 2H, CH_2, J = 7.2 Hz, CH_2); 3.06 (t, 2H, CH_2, J = 7.2 Hz); 3.62 (s, 3H, OCH_3); 7.45 (d, 1H, H_{arom}, J = 6.5 Hz); 7.59 (dd, 1H, H_{arom}, J = 6.5 Hz, J = 2.1 Hz); 7.74 (d, 1H, H_{arom}, J = 2.1 Hz). ^{13}C NMR: δ 28.19 (CH_2); 28.96 (CH_2); 51.46 (OCH_3); 117.94 (C-8); 127.27 (C-5); 128.73 (C-7); 129.97 (C-6); 131.32 (C-4a); 145.02 (C-8a); 152.12 (C═O); 158.19 (N═C); 172.49 (COO); ms: *m/z* 267 (M^+, 16%), 236 ($M^+ - OCH_3$, 15%), 208 ($M^+ - CO_2CH_3$, 5%), 180 ($M^+ - CH_2$-CH_2-CO_2CH_3, 100%). *Anal*. Calcd for $C_{12}H_{10}ClNO_4$: C, 53.85; H, 3.77; N, 5.23. Found: C, 53.83; H, 3.73; N, 5.16.

BIOLOGICAL ASSAYS

Antimicrobial activity of the substituted 3-(2-oxo-2*H*-benzo[*b*][1,4]oxazin-3-yl)propanoates (**3a–e**) was performed with the Bauer-Kirby method by the well diffusion technique using Petri plates made by Muller Hinton agar containing the culture [24,25]. This run involved the preparation of sterilized Whatman filter paper cut in form of disc of 0.6 cm of diameter, which was impregnated with the compound to be tested (160 mg/mL in DMSO), followed by adhering it to the surface of Petri plate that was previously inoculated with diluted culture. The incubation was realized at 37°C for 48 h for bacteria and at 25°C for 3–4 days for the yeast. The results, expressed as the inhibition aureola diameters in millimeter (mm) and the MIC (μg/mL), are shown in Table 1.

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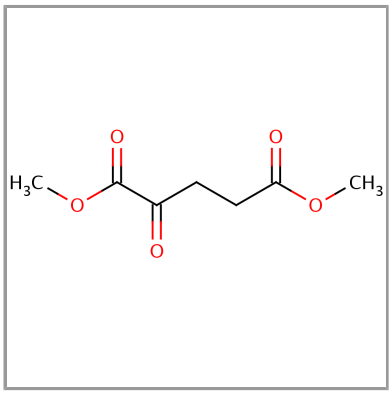

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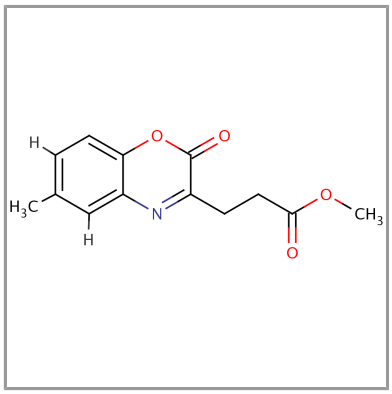

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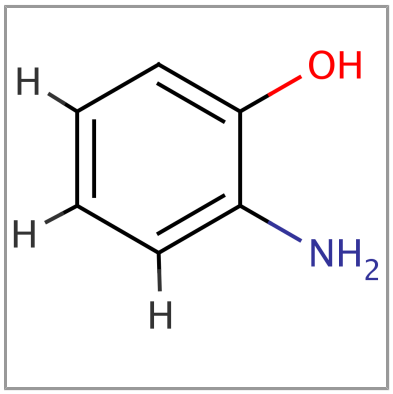

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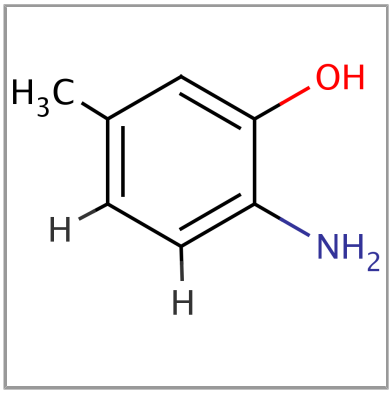

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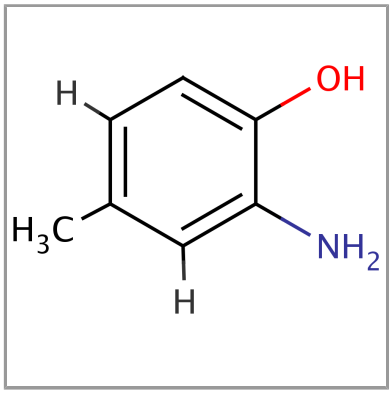

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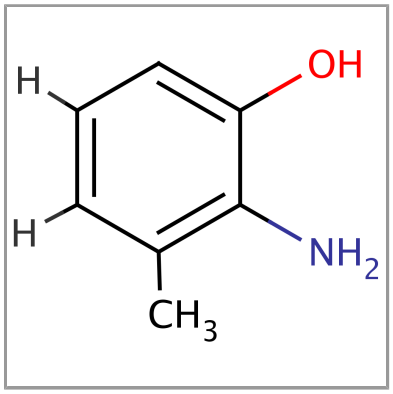

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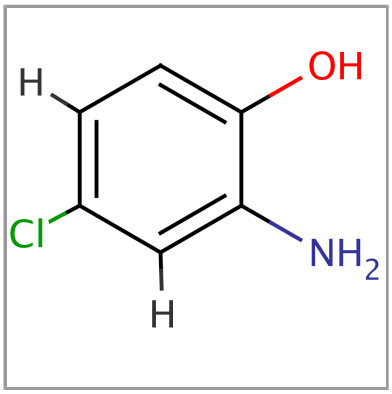

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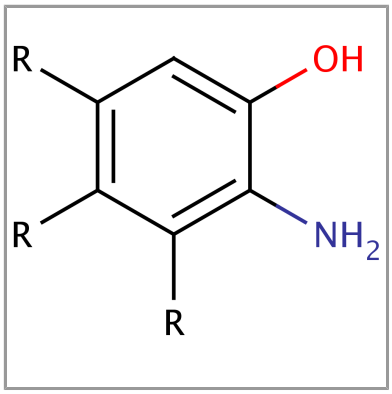

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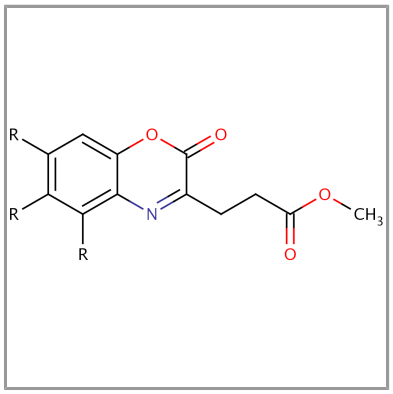
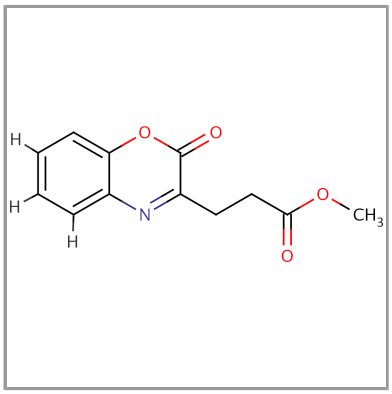


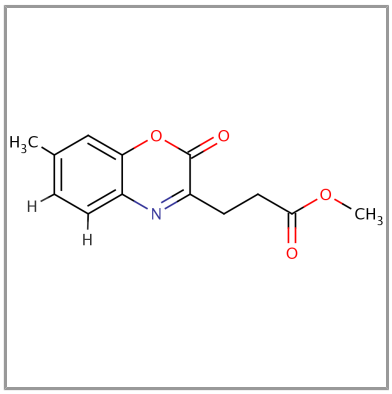
Compound Details Structure Search

3a



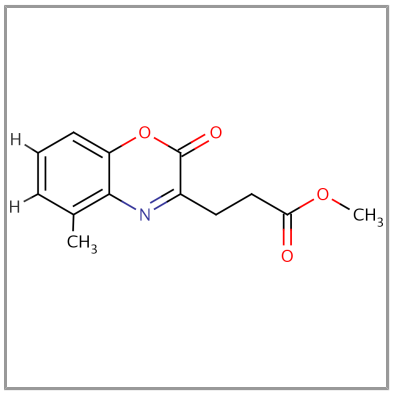
Compound Details Structure Search

3b



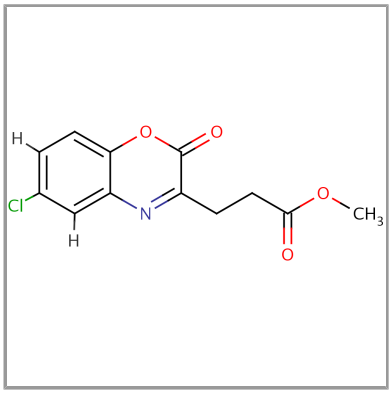
Compound Details Structure Search

3d



Compound Details Structure Search

3e



Compound Details Structure Search