EFFECT OF BROMINATION ON THE PHARMACOLOGICAL PROPERTIES OF METHYL 1-ALLYL-4-HYDROXY-2,2-DIOXO-1H-2λ6,1- BENZOTHIAZINE-3-CARBOXYLATE

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An x-ray crystal structure analysis found that the bromination of methyl 1-allyl-4-hydroxy-2,2-dioxo-1H-2λ6,1-benzothiazine-3-carboxylate by Br₂ in glacial HOAc involved not heterocyclization but classical addition of Br₂ to the unsaturated allyl bond, in contrast with structurally similar alkyl 1-allyl-4-hydroxy-2-oxo-1,2-dihydroquinoline-3-carboxylates. Pharmacological tests showed that removal of the double bond from the 1-N-alkyl moiety in the esters was associated with a decrease of analgesic activity.

Keywords: bromination, 4-hydroxy-2,1-benzothiazines, synthesis, analgesic activity.

The reaction of colored molecular Br₂ with an allyl double bond is well known in organic and analytical chemistry and can follow various pathways to form a variety of products. Thus, Br₂ adds totally to the unsaturated bond in the classical version [1]. However, in several instances this reaction occurs completely differently and is associated with exceedingly facile and effective formation of new 5-, 6-, and even 7-membered heterocycles [2 – 5]. Understandably, certain structural prerequisites in the molecule to be brominated are required for this. As a rule, these are cyclic compounds with ortho-allyl (or analogous) and carbonyl groups. Compelling examples of such compounds are 1-N-allyl-substituted quinolin-2-ones, bromination of which is insensitive to substituents in other positions of the quinolone core and always occurs primarily as a halocyclization. In fact, it is a preparative method for numerous 2-bromomethyl-1,2-dihydro-oxazolo[3, 2-a]quinolines [6 – 10].

Considering this and the close structural similarity of 4-R-2-oxo-1,2-dihydroquinoline-3-carboxylate esters, bromination of methyl 1-allyl-4-hydroxy-2,2-dioxo-1H-2λ6,1-benzothiazine-3-carboxylate (I) could be expected to form 5-oxo- (II) or 5-hydroxy- (III) tricyclic products depending on the capability for oxo–hydroxy tautomerism (Scheme 1).

However, it should be remembered that sometimes a new ring does not form even if all the criteria necessary for cyclization seem to be present [11, 12]. The true course of such reactions still cannot be predicted reliably so that they can be used for targeted modification of some derivatives or others. In other words, the bromination product of I could actually be 1-N-dibromopropyl derivative IV. We attempted to clarify in the present study what the actual product would be.

As it turned out, bromination of methyl 1-allyl-4-hydroxy-2,2-dioxo-1H-2λ6,1-benzothiazine-3-carboxylate (I) by Br₂ in glacial HOAc occurred readily and quickly. The brown Br₂ color faded practically instantaneously after mixing equimolar amounts of the reagents.

EXPERIMENTAL CHEMICAL PART

PMR spectra were recorded in DMSO-d₆ with TMS internal standard on a Varian Mercury-VX-200 spectrometer (USA) at operating frequency 200 MHz. Elemental analyses were performed on a EuroVector EA-3000 microanalyzer (Great Britain) and agreed with those calculated. Melting points were determined in capillaries on an SMP10 Stuart digital analyzer (Great Britain). Starting methyl ester I was prepared by the published method [13].

Methyl 1-(2,3-dibromopropyl)-4-hydroxy-2,2-dioxo-1H-2λ6,1-benzothiazine-3-carboxylate (IV). A solution of I (2.95 g, 0.01 mol) in glacial HOAc (10 mL) was stirred vigorously and treated with a solution of Br₂ (0.52 mL, 0.01 mol) in the same solvent (5 mL). The brown Br₂ color faded immediately. The reaction mixture was diluted with
cold H\textsubscript{2}O. The resulting precipitate was filtered off, rinsed with cold H\textsubscript{2}O, and dried. Yield 3.96 g (87%), mp 123 – 125°C (Et\textsubscript{2}O). PMR spectrum, δ, ppm (J, Hz): 13.21 (s, 1H, OH); 8.01 (d, 1H, J = 8.0, H-5); 7.76 (t, 1H, J = 7.7, H-7); 7.63 (d, 1H, J = 8.3, H-8); 7.41 (t, 1H, J = 7.6, H-6); 4.60 – 4.35 (m, 3H, NCH\textsubscript{2}CH\textsubscript{Br}); 3.95 (d, 2H, J = 4.0, CH\textsubscript{Br}); 3.88 (s, 3H, OMe). C\textsubscript{13}H\textsubscript{13}Br\textsubscript{2}NO\textsubscript{5}S.

The structure of the product formed by bromination of I was not so simple as first appeared to elucidate using NMR spectroscopy because all alternate structures (II, III, and IV) contained exactly the same spin systems. Therefore, it was impossible to solve the analytical problem using only ordinary PMR spectra. In principle, 2D NMR spectroscopy (HMOC and HMBC) could be used for this and are highly recommended for solving such problems [12]. However, the extremely unfortunate overlap of resonances that in this instance were important for assigning the NCH\textsubscript{2} protons and the CHBr moiety corresponding to it interfered with the practical use of these special NMR techniques.

Nevertheless, the structure of the obtained product was determined due to the formation during its purification of single crystals suitable for an x-ray crystal structure analysis. As a result, it was established unambiguously that bromination of I did not involve halocyclization, in contrast with the 2-carbonyl analogs, but ordinary addition of Br\textsubscript{2} to the allyl bond.

The dihydrothiazine heterocycle of the obtained methyl 1-(2,3-dibromopropyl)-4-hydroxy-2,2-dioxo-1H-2\textsubscript{a},1-benzothiazine-3-carboxylate (IV) had a conformation intermediate between a twist-boat and envelope (puckering parameters [14]: S = 0.64; θ = 51.8°; ψ = 23.4°). The deviations of S(1) and C(8) from the mean-square plane of the other ring atoms were 0.89 and 0.28 Å, respectively (Fig. 1). The N atom had a pyramidal configuration. The sum of the bond angles centered on it was 353°. The ester substituent was coplanar with the endocyclic C(7)–C(8) double bond [C(7)–C(8)–C(9)–O(2) torsion angle 4.2(8)°], which was assisted by the formation of a strong intramolecular H-bond O(1)–H…O(2) [H…O 1.61 Å, O–H…O 148°]. It is noteworthy that the formation of this H-bond did not redistribute electron density in the O(1)–C(7)–C(8)–C(9)–O(2) fragment, which is usually observed in structurally similar 4-hydroxyquinolin-2-ones. The substituent on the N atom was situated such that the C(12)–C(13) bond had the -sc-conformation relative to the C(1)–N(1) bond and antiperiplanar

Fig. 1. Molecular structure of IV represented as 50% probability thermal ellipsoids.
to the N(1)–C(11) bond. The torsion angles C(1)–N(1)–
C(11)–C(12) and N(1)–C(11)–C(12)–C(13) were 
–75.2(5)°
and 174.1(3)°, respectively. The Br atoms were located 
trans
relative to each other. The Br(1)–C(12)–C(13)–Br(2) torsion 
angle was 171.1(2)°. A short contact H(11a)...Br(2) of 2.83 
Å (sum of van-der-Waals radii 3.14 Å [15]) was observed in 
this substituent.

Rather strong steric repulsion between the dibromopro-
pyl moiety and the benzoazoxopyrimidine ring atoms (short intramo-
lecular contacts: H(2)...C(11) 2.55 Å (2.87 Å), H(2)... 
H(11a) 2.12 Å (2.34 Å), H(11a)...C(2) 2.65 Å (2.87 Å), 
H(11b)... O(5) 2.37 Å (2.46 Å), and H(12)...C(2) 2.75 Å 
(2.87 Å)] lengthened the C(1)...N(1) bond to 1.413(4) Å com-
pared with its average value of 1.371 Å [16]. Furthermore, an 
intermolecular C–H...n interaction C(11)...H(11a)...C(3)′ (1 
– x, 2 – y, –z) of 2.81 Å was observed in the crystal of ester 
IV.

X-ray structure analysis of IV. Crystals of IV 
(C_{13}H_{11}Br_{2}NO_{5}S, MW 455.12) were triclinic (Et_2O), at 20°C 
a = 8.384(1); b = 9.145(1); c = 11.246(2) Å; α = 68.13(1)°;
β = 89.11(1)°; γ = 71.14(1)°; V = 799.3(2) Å³; MW = 
455.12; Z = 2; space group PT; d_{calc} = 1.891 g/cm³; 
μ(MoKα) = 1.891 mm⁻¹; F(000) = 448. Unit-cell constants 
and intensities of 7947 reflections (4635 independent, 
R_{int} = 0.042) were measured on an Xcalibur-3 diffractometer 
(Mo Kα-radiation, CCD detector, graphite monochromator, 
ω-scanning, 2θ_{max} = 60°).

The structure was solved by direct methods using the 
SHELXTL programs [17]. Absorption was corrected semi-
empirically from multi-scanning results with 
T_{min} = 0.422 and 
T_{max} = 0.903. Positions of H atoms were found in differ-
ence electron-density syntheses and refined by a rider model 
with U_{iso} = nU_{eq} (n = 1.5 for methyl and 1.2 for other H at-
oms). H atoms involved in H-bonds were refined isotropi-
ally. The structure was refined over F² using anisotropic 
full-matrix least-squares methods for non-hydrogen atoms to 
R_{1} = 0.056 for 2521 reflections with F > 4σ(F), 
S = 0.932). The complete crystallo-
graphic dataset for IV was deposited in the Cambridge Cryst-
alllographic Data Centre (No. CCDC 982396).

EXPERIMENTAL BIOLOGICAL PART

Starting methyl ester I was shown before by us to pos-
sess high analgesic activity [13]. Hence, it seemed 
completely reasonable to study the analogical properties of its 
bromination product, i.e., ester IV. Tests were conducted on 
white laboratory mature male rats (180 – 200 g) according to 
requirements of the European Convention for the Protection 
of Vertebrate Animals used for Experimental and Other Sci-
entific Purposes and Ukrainian Law No. 3447-IV “On the 
Protection of Animals from Cruelty” (2006). Test animals 
were maintained during the whole study on a standard ration 
with free access to water and food.

Analgesic activity of IV was studied using a standard 
tail-immersion thermal irritation model that allowed the cen-
tral effect on the nociceptive system to be assessed [18]. The 
latent period in seconds for withdrawing the tail from a water 
bath heated to 54°C was measured after immersion. The 
analgesic effect (in %) was assessed from the latent period 1 h 
after administering the test compounds. Compound IV, the 
reference drugs, and the control were tested in seven test ani-
mals in order to obtain statistically significant results (signif-
icae level of confidence interval p (0.05). The test com-
 pound and structurally similar reference drugs piroxicam 
(Jenapharm, FRG) and meloxicam (Boehringer Ingelheim, 
FRG) were administered orally as thin aqueous suspensions 
stabilized by Tween-80 at a dose of 20 mg/kg. Control ani-
mals received an equivalent amount of water and Tween-80.

Table 1 presents the experimental results and shows that 
dibromo-substituted ester IV exhibited only a weak analgesic 
effect and was significantly less active than starting ester I, 
which was capable of prolonging the latent period by 71.1% 
relative to the control [13]. The allyl double bond could obvi-
ously be considered one of the important structural elements 
or pharmacophores mediating interaction with the biological 
target because other alkyl 1-R-4-hydroxy-2,2-dioxo-1H-
2λ₃,1-benzothiazine-3-carboxylates with ordinary alkyl sub-
stutents on the N atom did not exhibit significant analogical 
activity [13].

Although the 1-N-allyl→1-N-dibromopropyl conver-
sion performed by us did not improve the pharmacological 
properties of methyl 4-hydroxy-2,2-dioxo-1H-2λ₃,1-benzo-
thiazine-3-carboxylates; nevertheless, the established capa-
bility to convert 1-N-allyl-substituted 2,1-benzothiazines into 
1-N-(2,3-dibromopropyl) derivatives via bromination could 
turn out to be useful for chemical modification of the many 
N-R-amides of these acids or provide a platform for new 
promising pharmacological transformations involving the re-
active Br atoms.

** Differences statistically significant for p < 0.05 compared with es-
ter I;

| Compound     | Latent period 1 h after injection, s | Change of latent period compared with control, % |
|--------------|--------------------------------------|-----------------------------------------------|
| IV           | 3.53 ± 0.13*                         | + 12.6                                       |
| I            | 5.37 ± 0.18**                        | + 71.1                                       |
| Meloxicam    | 4.91 ± 0.17**                        | + 56.3                                       |
| Piroxicam    | 3.96 ± 0.15**                        | + 26.1                                       |
| Control      | 3.14 ± 0.14                          | –                                            |

* Differences statistically significant for p < 0.05 compared with es-
ter I;

** Differences statistically significant for p < 0.05 compared with the 
control.
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