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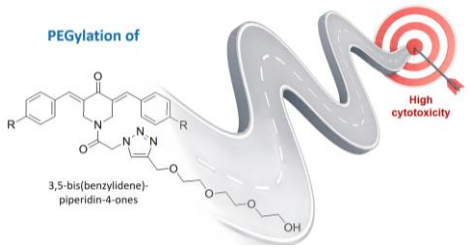
Triethylene glycol conjugation of 3,5-bis(benzylidene)piperidin-4-ones – a new approach to hybrid molecules with improved cytotoxic properties

Valery K. Brel,*[a] Margarita E. Neganova,[a] Yulia R. Aleksandrova,[a] Inna A. Shagina[a,b] and Valentina A. Karnoukhova[a]

[a] *A.N. Nesmeyanov Institute of Organoelement Compounds, Russian Academy of Sciences, 119334 Moscow, Russian Federation. E-mail: v_brel@mail.ru*
[b] *Institute of Physiologically Active Compounds, Federal Research Center of Problems of Chemical Physics and Medicinal Chemistry, Russian Academy of Sciences, 142432 Chernogolovka, Moscow Region, Russian Federation*

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Using the "click" chemistry methodology a series triethyleneglycol conjugation of 3,5-bis(benzylidene)-piperidin-4-ones was developed. It has been shown that introducing the 1,2,3-triazole–triethylene glycol molecular system into the structure of 3,5-bis(benzylidene)piperidin-4-ones leads to high submicromolar cytotoxicity against the majority of tumor cultures tested.



Keywords: synthesis, 3,5-bis(benzylidene)-piperidin-4-ones, hybrid molecules, cytotoxicity, cancer.

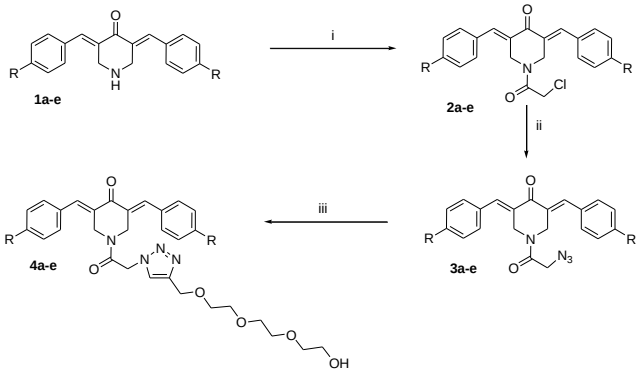
Curcumin has a wide range of biological activity and has been actively used in medical practice for many years[1]. Currently, curcumins with different carbon chain lengths, C-7 (dicarbonyl) and C-5 (monocarbonyl), have been isolated and described. Monocarbonyl analogues (MAC)[2] are widely used as effective scaffolds for the construction of hybrid molecules. The synthesized compounds using MAC, in particular 3,5-bis-(benzylidene)-piperidin-4-ones, demonstrate a wide range of biological activity, particularly on various cancer cell lines.[3,4] Various pharmacophore molecules, as well as compounds capable of improving the pharmacological profile of the resulting hybrid molecule, can serve as partners for conjugation with MAC.[5]

In this study, we have demonstrated for the first time that the creation of MAC conjugates with polyester fragments has a significant impact on the cytotoxic properties of the resulting hybrid structures. The conjugation of biological substrates with polyester modifiers is a well-known technique and is used to enhance the pharmacological profile of biological substrates. The conjugation of biological substrates with polyester modifiers is a well known technique and is used to enhance the pharmacological profile of biological substrates. For example, the use of polyesters leads to improved pharmacokinetic properties of peptide and protein based medicinal products.[6,7]

The starting compounds for triethyleneglycol functionalization were selected as (3*E*,5*E*)-1-(2-azidoacetyl)-3,5-bis(benzylidene)-piperidin-4-ones, the synthesis of which was previously published by us. In this work, we report the details of the synthesis of a series of new hybrid molecules obtained on the basis of 3,5-bis-(benzylidene)-piperidin-4-ones, the study of the structure of the obtained compounds using NMR and X-ray crystallographic analysis, and biological screening for cytotoxicity. To create covalently linked hybrids **4a–e**, we used a "click" chemistry methodology that we had previously employed for the design of other biologically active substrates.[8] This approach not only enables the conjugation of triethylene glycol with 3,5-bis-(benzylidene)-piperidin-4-ones but also allows for the additional introduction of a 1,2,3-triazole moiety, which may influence the pharmacological profile of conjugates **4a–e**. The synthesis of compounds **4a–e** involves three stages: the preparation of (3E,5E)-1-(2-chloroacetyl)-3,5-bis(benzylidene)-piperidin-4-ones **2a–e**, followed by azidation to (3E,5E)-1-(2-azidoacetyl)-3,5-bis(benzylidene)-piperidin-4-ones **3a–e**, and the synthesis of the target compounds **4a–e**. The synthesis of compounds **2a-e** and **3a-e** was carried out using a previously described method.[9] Reactions of azides **3a-e** was performed at room temperature in DCM under [2+3]-cycloaddition conditions, using CuBr as a catalyst. As an acetylene reagent for carrying out the "click" chemistry reaction, mono-propargyl ether of triethylene glycol was used. The reaction progress was monitored using TLC. The reaction was carried out at room temperature in DCM until the starting compounds disappeared.

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The structure of **3a-e** and **4a–e** was confirmed using ^{1}H and ^{13}C NMR spectroscopy. (Details of the synthesis of compounds **4a–e** and ^{1}H and ^{13}C NMR spectra see Supplementary Materials).



R=H (**a**); R=F(**b**); R=Cl,(**c**); R=OCH_3,(**d**); R=$C(CH_3)_2$ (**e**).

Scheme 1 *Reagents and conditions*: i, $ClCH_2COCl$, CH_2Cl_2, r.t., 2 h; ii NaN_3, CH_3CN, 80^0 C; iii, $HO(CH_2CH_2O)_3CH_2C{\equiv}CH$, CuBr, DIPEA, r.t., 24h.

The structure of molecule **4c** was confirmed by single crystal X-ray diffraction analysis (Figure 1).[†] All bond lengths and angles are within the expected ranges, as confirmed by the Mogul geometry check[13].

The central six-membered heterocycle adopts a sofa conformation with the N1 atom deviated by 0.666 Å from the mean plane of the essentially planar C1–C5 part (the maximal deviation 0.041 Å). The N1 atom has a slightly pyramidal configuration with the sum of bond angles 357.2°. The interplane angle between the triazole ring and the mean plane C1–C5 is 71.94°. Both olefinic double bonds have an *E* configuration which is typical for this class of compounds. One of the exocyclic carbon atoms C6 is displaced by 0.200 Å from the mean plane C1–C5, while the second atom C13 lies virtually in the plane (0.035 Å). Both aryl rings are rotated relative to the olefinic double bonds with the torsion angles C2–C6–C7–C12 and C5–C13–C14–C19 equal to –16.62 and 31.27°, respectively. This non-coplanarity can be attributed, at least in part, to non-bonded interactions between the equatorial protons connected to C3 and C4 with the *ortho* hydrogen atoms attached to atoms C12 and C19[13]. The bond angles C2–C6–C7 and C5–C13–C14 (130.9(3) and 128.2(3)°) significantly greater than 120° reduces this steric repulsion.

[†]*Crystal data for* **4c.** $C_{30}H_{32}Cl_2N_4O_6$ (*M* = 615.49), monoclinic, space group $P2_1/c$, *a* = 7.7462(6), *b* = 22.8298(18) and *c* = 16.6091(13) Å, *β* =99.560(3)°, *V* = 2896.4(4) Å^3, *Z* = 4, d_{calc} = 1.411 g·cm^{-3}, μ(MoKα) = 0.275 mm^{-1}. The single crystal XRD experiment was performed at 120 K on a Bruker SMART APEX II diffractometer equipped with a Photon II detector using a standard procedure (graphite monochromated MoKα radiation, φ- and ω-scanning). Within the angles 4.35 <2*θ*< 52°, a total of 29056 reflections were measured, 5689 unique reflections (*R*int = 0.1232), 3017 reflections with $I > 2\sigma(I)$. The structure was solved with the SHELXT[10] program using Intrinsic Phasing and refined by the full-matrix least-squares technique against F^2_{hkl} in anisotropic approximation for non-hydrogen atoms with the SHELXL[11] program incorporated in the OLEX2 program package[12]. The hydrogen atom of the OH-group was found from difference Fourier synthesis and refined isotropically. Other hydrogen atoms were placed in calculated positions and refined in the riding model. GOOF = 1.019; the final *R* values: R_1 = 0.0544 [$I > 2\sigma(I)$],wR_2 = 0.1221 (all data). Residual electronic density max/min was 0.327/ –0.361 eÅ^{-3}.
CCDC 2583556 contains the supplementary crystallographic data forthis paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

The flexible triethylene glycol fragment does not adopt an extended conformation away from the central heterocyclic part, but instead folds towards the amide oxygen atom and one of the aryl rings making the molecular shape more compact. This conformation is stabilized by intramolecular CH···π, CH···O and CH···Cl interactions.

The main features of crystal packing are H-bonded chains (Figure S1) formed by terminal OH groups of the flexible fragments and N atoms of the triazole rings (O6–H6···N4 (2.851(4) Å; O-H···N 162.43°, with O–H distance normalized to 0.993 Å), and stacks of hydrophobic conjugated systems forming geometrical layers alternating with layers of more hydrophilic molecular fragments (Figure S2).

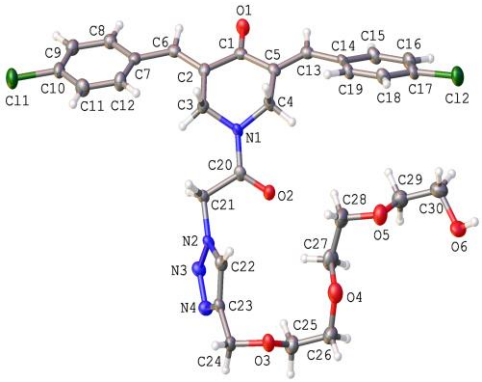


Figure 1 General view of **4c** in crystal in thermal ellipsoid representation (p = 50%). For selected bond lengths and angles, see Online Supplementary Materials.

Evaluation of the biological activity of the synthesized compounds (**4a–e**) demonstrated their pronounced antitumor efficacy and made it possible to identify clear patterns of structure–activity relationships.

Table 1. IC_{50} of the 3,5-bis(benzylidene)-piperidin-4-ones **3a-e**[9] and **4a-e**, 72-hours incubation with different lines of cells.

Comp.	IC_{50} (μM), 72 h				
	T24	SH-SY5Y	T98G	A431	Hek-293
3a	24.87±0.86	-	9.55 ± 0.17	7.66 ± 0.01	7,12 ± 0,59
3b	7.59 ± 0.34	-	8.05 ± 0.04	6.29 ± 0.23	6,95 ± 0,03
3c	6.62 ± 0.17	-	7.36 ± 0.09	5.64 ± 0.20	5,59 ± 0,23
3d	14.74 ± 0.25	-	9.89± 0.19	8.32 ± 0.04	7,84 ± 0,01
3e	7.45 ± 2.63	-	8.03 ± 0.01	4.50 ± 0.03	8,77 ± 0,21
4a	7,52±0,18	4,59±0,12	21,55±0,04	7,29±0,06	6,41±0,02
4b	0,82±0,01	0,59±0,03	7,53±0,22	0,93±0,01	0,79±0,01
4c	0,69±0,01	0,50±0,02	7,07±0,03	0,80±0,01	0,85±0,01
4d	7,68±0,03	5,44±0,12	8,29±0,27	7,93±0,27	7,42± 0,17
4e	5,44±0,09	5,85±0,04	6,41±0,09	8,19±0,26	6,76± 0,33

The data are presented as IC_{50} results in μM — the concentration at which 50% of cells die.

First of all, it is worth noting that the introduction of halogen substituents into the structure of the base molecule led to a sharp and multiple increase in the cytotoxic effect compared to the unsubstituted analogue **4a** (R=H), as well as derivatives containing methoxy (**4d**) and isopropyl (**4e**) groups. The most

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outstanding results were shown by compounds **4b** and **4c**, which showed high submicromolar cytotoxicity against the majority of tumor cultures tested. Among the cell lines studied, neuroblastoma SH-SY5Y and bladder carcinoma T24 cells demonstrated the greatest sensitivity to the effects of halogenated derivatives. Also, when comparing the data with previous work[9], it is obvious that introducing the 1,2,3-triazole–triethylene glycol molecular system into the structure of 3,5-bis(benzylidene)-piperidin-4-ones led to significant an improvement in cytotoxic properties.

The results obtained convincingly demonstrate that the chosen strategy of synthetic modification of 3,5-bis(benzylidene)-piperidin-4-ones, using the “click” - chemistry methodology and the targeted introduction of halogens (fluorine and chlorine), made it possible to obtain significantly more active chemical compounds with high potential for further development as a basis for creating effective cytotoxic agents.

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Crystal structure determination of **4c** was performed employing the equipment of the Center for Collective Use of INEOS RAS.

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.8195.

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