SYNTHESIS AND THE BIOLOGICAL ACTIVITY OF 4-HYDROXY-2,2-DIOXO-1H-2λ,1-BENZOTHIAZIN-3-CARBOXYLIC ACIDS TRIFLUOROMETHYL-SUBSTITUTED ANILIDES

L.A. Petrushova, I.V. Ukrainets, S.P. Dzyubenko*, L.A. Grinevich

National University of Pharmacy
61002, Kharkiv, 53 Pushkinska str. E-mail: uiv-2@mail.ru

Key words: anilides; 2,1-benzothiazines; synthesis; trifluoromethyl group; analgesic activity; diuretic properties

* National Pirogov Memorial Medical University
Due to its powerful electron-withdrawing properties the trifluoromethyl group is able to enhance reactivity of various electrophilic substrates and affect regioselectivity of reactions involving nucleophiles [1-2]. The presence of this substituent often allows to carry out chemical transformations easily that in its absence do not proceed even in the most rigid conditions; thus, it is widely used in modern preparative organic synthesis. By its intensive development chemistry of trifluoromethylated compounds is obliged to the complex of the desirable properties acquired, such as resistance to external factors – temperature, sunlight, oxidation, etc.

The unique effect of the trifluoromethyl substituents on the properties of the molecule as a whole has not remained without attention of medicinal chemistry – their ability to change significantly the interaction of the active ingredient with the target molecule, peculiarities of its metabolism and other pharmacodynamic and/or pharmacokinetic characteristics has been effectively used for a long time when creating new biologically active substances [3]. As a result, the range of drugs currently used is more than three dozen drugs of different pharmacological groups containing one or even a few of the trifluoromethyl fragments in their structure [4]. It is not surprising that this methodology has not lost its significance, and it is used very widely by modern medicinal chemistry [5-12].

Taking into account these circumstances and in continuation of our studies on the methods of synthesis, chemical and biological properties of derivatives of 2,1-benzothiazines, this message is devoted to trifluoromethyl analogues of the methyl-substituted 4-hydroxy-1-methyl-2,2-dioxo-1H-2λ6,1-benzothiazin-3-carboxanilides described earlier [13].

Theoretically, there are several ways of direct trifluoromethylation of organic compounds [14-21]. However, it is quite clear that to obtain the objects of the present study it is advisable to use commercially available mon trifluoromethanilides; in their reaction with esters (1) in a boiling xylene the synthesis of the corresponding 1-R-4-hydroxy-1-methyl-2,2-dioxo-1H-2λ6,1-benzothiazin-3-carboxanilides 2a-f has been carried out with good yields and purity (Scheme).

Anilides 2a-f are white to yellowish white crystalline substances with the narrow melting temperature range (Table 1). They are moderately soluble in DMF, DMSO and ethyl acetate at room temperature, and sparingly soluble in alcohol, are practically insoluble in water, ether and hexane, but readily soluble in hot aqueous alkaline solutions. Their structure was confirmed by elemental analysis and NMR 1H spectroscopy (Tab. 1).

A powerful electron withdrawing effect exhibited by trifluoromethyl groups on the carbon atoms of arylamide moieties, and hence to the protons bound with them, is bound to be reflected in the 1H NMR spectra of anilides 2a-f. Indeed, compared with the spectra of the model methyl derivatives, resonance signals of the corresponding anilide protons in the spectra of their fluoromethyl analogues 2a-f undergo a significant paramagnetic shift. Aromatic protons of benzothiazine rings are too far from trifluoromethyl substituents to be affected by them in such a noticeable extent. Therefore, their signals practically do not change their positions in the 1H NMR spectra in going from methyl derivatives to trifluoromethyl ones (see Fig.).

Analgesic properties of the trifluoromethanilides 2a-f synthesized were studied in white outbred adult male rats weighing 180-200 g in full compliance with the provisions of the European Convention on Protection of Vertebrates Used for Experimental and Other Scientific Purposes and the Ukrainian Law No. 3447-IV "On protection of animals from severe treatment" (2006).

The standard model of the tail-flick thermal stimulation was used in the study. It allows identifying the central component affecting the nociceptive system in the mechanism of the analgesic effect of the substances studied [22]: the rat’s tail tip was immersed in a water bath heated to 54°C, after that the latent period of the tail withdrawal (immersion) expressed in seconds was determined. The substances under research and their structurally similar reference drugs – Meloxicam and Piroxicam – were introduced orally in the form of fine aqueous suspensions stabilized with Tween-80 in the screening dose of 20 mg/kg. The control group received an equivalent amount of water with Tween-80. The analgetic effect (in %) was assessed by the change of the latent period in 1 hour after administration of the test substances.
Seven experimental animals were involved to obtain statistically reliable results of each trifluoromethyl anilide 2a-f, reference drugs in testing and control. The results of biological tests were processed by the method of variation statistics using Student’s t-criterion.

The comparative analysis of the experimental data given in Tab. 3 with the results of the previous studies [13] shows that the replacement of the methyl group in the anilide moiety of 1-R-4-hydroxy-2,2-dioxo-1H-2λ6,1-benzothiazin-3-carboxamides to the trifluoromethyl one affects quite ambiguously the ability to suppress the pain reaction. In the case of anilide 2a it has no effect on this ability, but in the cases of anilides 2b, d, f it leads to its complete loss. At the same time there are some positive examples, in particular, trifluoromethyl substituted anilides 2c,e show a significant increase in activity compared to their methyl analogues [13]. According to the level of the analgesic effect revealed they significantly exceed Piroxicam, and are almost as good as Meloxicam.

The effect of trifluoromethyl anilinilides 2a-f on the urinary function of the kidneys was studied according to the classical method [23] on white outbred rats of both sexes weighing 180-200 g in parallel and compared with Hydrochlorothiazide. All animals received water load in the amount of 25 ml/kg by gavage. The compounds studied were administered orally in the form of a thin aqueous suspension stabilized with Tween-80 in the screening dose of 10 mg/kg, and Hydrochlorothiazide was taken in its effective dose (40 mg/kg). The control group received only simi-

### Table 1

| Compound | Empirical formula | Found, % / Calculated, % | Mp, °C | Yield, % |
|----------|------------------|--------------------------|--------|----------|
| 2a       | C16H11F3N2O4S    | 49.94 / 50.00 2.80 / 2.88 7.37 / 7.29 8.26 / 8.34 | 181-182 | 86       |
| 2b       | C16H11F3N2O4S    | 49.92 / 50.00 2.95 / 2.88 7.35 / 7.29 8.23 / 8.34 | 226-228 | 88       |
| 2c       | C16H11F3N2O4S    | 50.09 / 50.00 2.97 / 2.88 7.38 / 7.29 8.25 / 8.34 | 245-247 | 92       |
| 2d       | C17H13F3N2O4S    | 51.35 / 51.26 3.37 / 3.29 6.95 / 7.03 7.96 / 8.05 | 157-159 | 85       |
| 2e       | C17H13F3N2O4S    | 51.33 / 51.26 3.34 / 3.29 6.97 / 7.03 7.95 / 8.05 | 143-145 | 91       |
| 2f       | C17H13F3N2O4S    | 51.31 / 51.26 3.36 / 3.29 7.08 / 7.03 7.99 / 8.05 | 184-186 | 94       |

Fig. Fragments of the 1H NMR spectra (signals of aromatic protons) of 4-methyl anilide of 4-hydroxy-1-methyl-2,2-dioxo-1H-2λ6,1-benzothiazin-3-carboxylic acid [13] and its 4-trifluoromethyl substituted analogue 2f.
lar amount of water with Tween-80. After that the experimental animals were placed in “metabolism cages”. The amount of urine excreted by the animals within 4 hours was the indicator of the intensity of uropoiesis. The results obtained (Table 4) show that when transferring from the methyl derivatives to trifluoromethyl ones the ability of N-aryl-4-hydroxy-2,2-dioxo-1H-2λ1,1-benzothiazin-3-carboxamide to increase or, conversely, inhibit diuresis is completely lost. Therefore, in the search for new potential diuretics our chemical modification is impractical.

**Experimental Part**

1H NMR spectra of the compounds synthesized were recorded on a Varian Mercury-400 device (with the operating frequency of 400 MHz) in the solution of DMSO-d$_6$, the TMS internal standard. Elemental analysis was performed by an EuroVector EA-3000 microanalyser. Melting points were determined in the capillary on a SMP10 Stuart digital analyzer of the melting point. The initial esters of 1-R-4-hydroxy-1-methyl-2,2-dioxo-1H-2λ,1-benzothiazin-3-carboxylate (3) were synthesized by the method described earlier [24].

**Table 2**

1H NMR Spectra of trifluoromethyl-substituted anilides 2a-f

| Compound | Chemical shifts, δ ppm (J, Hz) |
|----------|---------------------------------|
| 2a       | 15.15 (1H, br. s, OH); 12.28 (1H, br. s, SO$_3$N$_2$); 9.63 (1H, s, CONH); 8.03-7.98 (2H, m, H-5.6'); 7.75 (1H, d, J = 7.8, H-3'); 7.70 (1H, t, J = 7.7, H-4'); 7.64 (1H, t, J = 7.7, H-7); 7.46 (1H, t, J = 7.7, H-5'); 7.28 (1H, t, J = 7.7, H-6); 7.22 (1H, d, J = 8.3, H-8) |
| 2b       | 15.18 (1H, br. s, OH); 12.30 (1H, br. s, SO$_3$N$_2$); 9.71 (1H, s, CONH); 8.06 (1H, s, H-2'); 8.01 (1H, d, J = 7.9, H-5); 7.80 (1H, d, J = 7.9, H-6); 7.63 (1H, t, J = 7.6, H-7); 7.58 (1H, t, J = 7.9, H-5'); 7.47 (1H, d, J = 7.5, H-4'); 7.28 (1H, t, J = 7.6, H-6); 7.21 (1H, d, J = 8.3, H-8) |
| 2c       | 15.16 (1H, br. s, OH); 12.28 (1H, br. s, SO$_3$N$_2$); 9.79 (1H, s, CONH); 8.00 (1H, d, J = 7.9, H-5); 7.84 (2H, d, J = 7.9, H-2'); 7.67 (2H, d, J = 8.0, H-3',5'); 7.62 (1H, t, J = 7.7, H-7); 7.27 (1H, t, J = 7.6, H-6); 7.21 (1H, d, J = 8.1, H-8) |
| 2d       | 14.93 (1H, br. s, OH); 9.66 (1H, s, CONH); 8.09 (1H, d, J = 7.9, H-5); 7.99 (1H, d, J = 8.0, H-2'); 7.78 (1H, t, J = 7.9, H-4'); 7.74 (1H, d, J = 7.9, H-3'); 7.70 (1H, t, J = 7.9, H-7); 7.49 (1H, d, J = 8.4, H-8); 7.44 (1H, t, J = 7.9, H-5'); 7.39 (1H, t, J = 7.5, H-6); 3.51 (3H, s, N-Me) |
| 2e       | 14.98 (1H, br. s, OH); 9.82 (1H, s, CONH); 8.09 (1H, d, J = 7.9, H-5); 8.05 (1H, s, H-2'); 7.81 (1H, d, J = 7.9, H-6'); 7.76 (1H, t, J = 7.8, H-7); 7.59 (1H, t, J = 7.9, H-5'); 7.50-7.45 (2H, m, H-8,4'); 7.38 (1H, t, J = 7.6, H-6); 3.50 (3H, s, N-Me) |
| 2f       | 15.02 (1H, br. s, OH); 9.80 (1H, s, CONH); 8.08 (1H, d, J = 7.9, H-5); 7.83 (2H, d, J = 8.0, H-2'); 7.77 (1H, t, J = 7.7, H-7); 7.66 (2H, d, J = 8.0, H-3',5'); 7.48 (1H, d, J = 8.3, H-8); 7.38 (1H, t, J = 7.4, H-6); 3.50 (3H, s, N-Me) |

**Table 3**

The analgesic properties of anilides 2a-f on the “tail-flick” model in rats

| Compound | R | Position CF$_3$ | The latent period in 1 h after administration of the compounds, s | Change of the latent period, compared to control, % |
|----------|---|-----------------|---------------------------------------------------------------|--------------------------------------------------|
| 2a       | H | 2               | 3.14±0.11                                                      | 0                                                |
| 2b       | H | 2               | 3.15±0.10                                                      | 0                                                |
| 2c       | H | 4               | 4.84±0.14                                                      | +54.0                                            |
| 2d       | Me| 2               | 3.69±0.12                                                     | +17.6                                            |
| 2e       | Me| 3               | 4.50±0.15                                                     | +43.2                                            |
| 2f       | Me| 4               | 3.40±0.12                                                     | +8.3                                             |
| Meloxicam | - | -               | 4.91±0.17                                                     | +56.3                                            |
| Piroxicam | - | -               | 3.96±0.15                                                     | +26.1                                            |
| Control  | - | -               | 3.14±0.14                                                      | -                                                |

* Differences were significant at p<0.05 compared to the control.

**Table 4**

The diuretic activity for anilides 2a-f and Hydrochlorothiazide

| Compound | Diuresis within 4 h, ml | Diuretic activity * % |
|----------|-------------------------|-----------------------|
| 2a       | 4.04±0.26               | -5                    |
| 2b       | 4.89±0.32               | +15                   |
| 2c       | 4.77±0.30               | +12                   |
| 2d       | 3.96±0.25               | -7                    |
| 2e       | 4.38±0.31               | +3                    |
| 2f       | 4.56±0.35               | +7                    |
| Hydrochlorothiazide | 6.43±0.38** | +51 |
| Control  | 4.26±0.33               | -                     |

* “+” – increase, “–” – inhibition of diuresis compared to the control taken as 100%; ** – differences were significant at p<0.05 compared to the control.
washed with cold ethanol and dried. Crystallize from the mixture of DMF/ethanol.

**Conclusions**

1. Trifluoromethyl-substituted anilides of 4-hydroxy-2,2-dioxo-1H-2λ,1-benzothiazin-3-carboxylic acids have been synthesized in order to identify the structural and biological regularities that are important for the subsequent search for new analgesics and diuretics in a series of 2,1-benzothiazine.

2. As a result of the pharmacological screening it has been found that the presence of trifluoromethyl groups in the anilide moiety of N-aryl-4-hydroxy-2,2-dioxo-1H-2λ,1-benzothiazin-3-carboxamides positively affects their analgesic properties, but it does not provide the diuretic activity.

**References**

1. O’Connor M. J., Boblak K. N., Topinka M. J., Kindelin P. J., Briski J. M., Zheng C., Klumpp D. A. Journal of the American Chemical Society, 2010, Vol. 132, No.10, pp.3266-3267. DOI: 10.1021/ja101482j.

2. True J. E., T. D., Winter R. W., Gard G. L. Inorganic Chemistry, 2003, Vol. 42, No.14, pp.4437-4441. DOI: 10.1021/ic034329h.

3. Yule H. L. Journal of Medicinal and Pharmaceutical Chemistry, 1959, Vol. 1, No.2, pp.121-133. DOI: 10.1021/jm50003aa001.

4. Kleemann A., Engel J., Katscher B., Reichert D. Pharmaceutical Substances: Syntheses, Patents, Applications of the most relevant APIs, 5-th ed. Stuttgarg, Thieme, 2008.

5. Luzina E. L., Popov A. V. Journal of Fluorine Chemistry, 2014, Vol. 168, pp.121-127.

6. Pasquier B., El-Ahmad Y., Filoche-Romètre B., Dureuil-Sizaire C., Fassy F., Abecassis P. Y., Mathieu M., Bertrand T., Benard T., Barrière C., El Batti S., Letallec J. P., Sonnefraud V., Brollo M., Delbarre L., Loyau V., Pilorge F., Bertin L., Richepin P., Arigon J., Labrosse J. R., Clément J., Durand F., Combet R., Perraut P., Leroy V., Gay F., Lefrancois D., Breton E., Marquette J. P., Michot N., Caron A., Castell C., Schio L., McCort G., Goulouc H., Garco-Echeverria C., Ronan B. J. Journal of Pharmaceutical and Medicinal Chemistry, 2015, Vol. 58, No.1, pp.376-400. DOI: 10.1021/jp5013352.

7. Wang Z., Sims C. R., Patil N. K., Godden N., Mayeux P. R. The Journal of Pharmacology and Experimental Therapeutics, 2015, Vol. 352, No.1, pp.61-66. DOI: 10.1124/jpet.114.219394.

8. Raffi G., Khan G., Vanhoutte P. M. The Journal of Pharmacology and Experimental Therapeutics, 2015, Vol. 352, No.1, pp.14-22. DOI: 10.1124/jpet.114.217935.

9. Birrell G. W., Chavchich M., Ager A. L., Shieh H. M., Heffernan G. D., Zhao W., Krasucki P. E., Saizoz K. W., Terpinjski J., Schieberer C. A., Jacobsu L. R., Shanks G. D., Jacobus D. P., Edstein M. D. Antimicrobial Agents and Chemotherapy, 2014, Vol. 59, No.1, pp.70-7. DOI: 10.1128/AAC.03762-14.

10. Mathys M., Kraft P. Chemistry & Biodiversity, 2014, Vol. 11, No.10, pp.1597-607. DOI: 10.1002/cbdv.201400011.

11. Abdellatif K. R., Moawad A., Knas E. E. Bioorganic & Medicinal Chemistry Letters, 2014, Vol. 24, No.21, pp.5015-5021. DOI: 10.1016/j.bmcl.2014.09.024.

12. Moreno-Rodríguez A., Salazar-Schettino P. M., Bautista J. L., Hernández-Luis F., Torres H., Guevara-Gómez Y., Pina-Canseco S., Torres M. B., Cabrera-Braun M., Martínez C. M., Pérez-Campes E. European Journal of Medicinal Chemistry, 2014, Vol. 87, pp.23-29. DOI: 10.1016/j.ejmech.2014.09.027.

13. Ukrainets I. V., Petrushova L. A., Dzyubenko S. P. Zhurnal Organichnoi ta Farmatsevtichnoi Khimii – Journal of Organic and Pharmaceutical Chemistry, 2014, Vol. 12, No.2(46), pp.53-58.

14. Bravo M., Martinez C. M., Pérez-Campos E. European Journal of Medicinal Chemistry, 2014, Vol. 87, pp.23-29. DOI: 10.1016/j.ejmech.2014.09.027.

15. Mathys M., Kraft P. Chemistry & Biodiversity, 2014, Vol. 11, No.10, pp.1597-607. DOI: 10.1002/cbdv.201400011.

16. Moren-Rodriguez A., Salazar-Schettino P. M., Bautista J. L., Hernández-Luis F., Torres H., Guevara-Gómez Y., Pina-Canseco S., Torres M. B., Cabrera-Braun M., Martínez C. M., Pérez-Campes E. European Journal of Medicinal Chemistry, 2014, Vol. 87, pp.23-29. DOI: 10.1016/j.ejmech.2014.09.027.

17. Ukrainets I. V., Petrushova L. A., Dzyubenko S. P. Chemistry of Heterocyclic Compounds, 2013, Vol. 49, No.9, pp.1378-1383.

18. Stahly G. P., Bell D. R. The Journal of Organic Chemistry, 1989, Vol. 54, No.12, pp.2873-2877. DOI: 10.1021/jo00273a020.

19. Raffi G., Khan G., Vanhoutte P. M. The Journal of Pharmacology and Experimental Therapeutics, 2015, Vol. 352, No.1, pp.14-22. DOI: 10.1124/jpet.114.217935.

20. Moren-Rodriguez A., Salazar-Schettino P. M., Bautista J. L., Hernández-Luis F., Torres H., Guevara-Gómez Y., Pina-Canseco S., Torres M. B., Cabrera-Braun M., Martínez C. M., Pérez-Campe E. European Journal of Medicinal Chemistry, 2014, Vol. 87, pp.23-29. DOI: 10.1016/j.ejmech.2014.09.027.

21. Ukrainets I. V., Petrushova L. A., Dzyubenko S. P. Chemistry of Heterocyclic Compounds, 2013, Vol. 49, No.9, pp.1378-1383.

Надійшла до редакції 15.01.2015 р.