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Synthesis and Antimicrobial Activity of *N*-(Indolyl)trifluoroacetamides

I. S. Stepanenko[a], S. A. Yamashkin[b, *], A. I. Kot'kin[b], and M. A. Yurovskaya[c]

[a] *Ogarev Mordovia State University, Saransk, Russia*
[b] *Evseviev Mordovian State Pedagogical Institute, Saransk, Russia*
[c] *Department of Organic Chemistry, Moscow State University, Moscow, Russia*
**e-mail: yamashk@yandex.ru*

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Abstract—A method for the synthesis of the corresponding trifluoroacetamides based on 2,3-dimethyl-, 1,2,3-trimethyl-7-aminoindoles and ethyl ester of trifluoroacetic acid is developed. The compounds obtained are screened for antimicrobial activity using the standard strains of the *Staphylococcus aureus* 29213, *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, *Streptococcus pyogenes* 1238, and *Klebsiella pneumonia* 9172; and the antimicrobial activity comparable to dioksidin, a widely used antimicrobial drug, is demonstrated.

Keywords: The reaction of heteroaromatic amines with ethyl ether of trifluoroacetic acid, the synthesis of *N*-(indolyl)-2,2,2-trifluoroacetamides, antimicrobial activity of *N*-(indolyl) trifluoroacetamides

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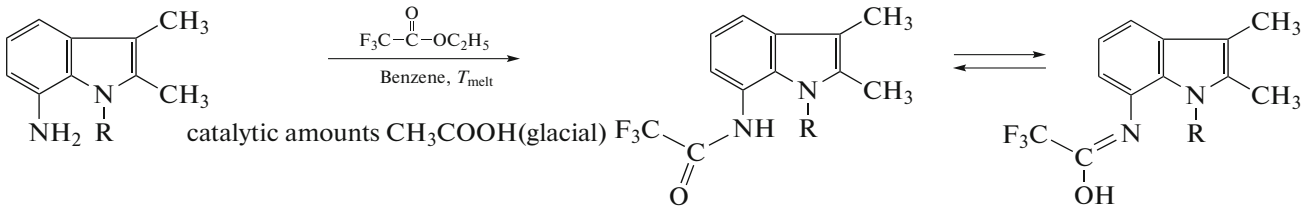
Substituted aminoindoles with an amino group in the benzene ring are well known as starting compounds for the production of indolyl amides, indolylene amino ketones, pyrroloindoles, and pyrroloquinolones [1, 2]. Many of these compounds have a different biological activity [3–5]. Indolyl amides, pyrroloquinolones based on substituted 4,7-aminoindoles and trifluoroacetoacetic ester have a high level of antimicrobial activity [6, 7]. In this regard, it was interesting to study the reaction of 2,3-dimethyl- and 1,2,3-trimethyl-7-aminoindoles (1, **2**) with trifluoroacetic acid ethyl ester in order to develop a method for the synthesis of the corresponding indolyl trifluoroacetamides and their antimicrobial activity.

We found that heating aminoindoles **1** and **2** with an excess of trifluoroacetic acid ethyl ester in benzene in the presence of a catalytic amount of glacial acetic acid made it possible to produce compounds, which, according to the mass spectral studies (UV, ^{1}H NMR), have structures **3** and **4** shown in Scheme 1.



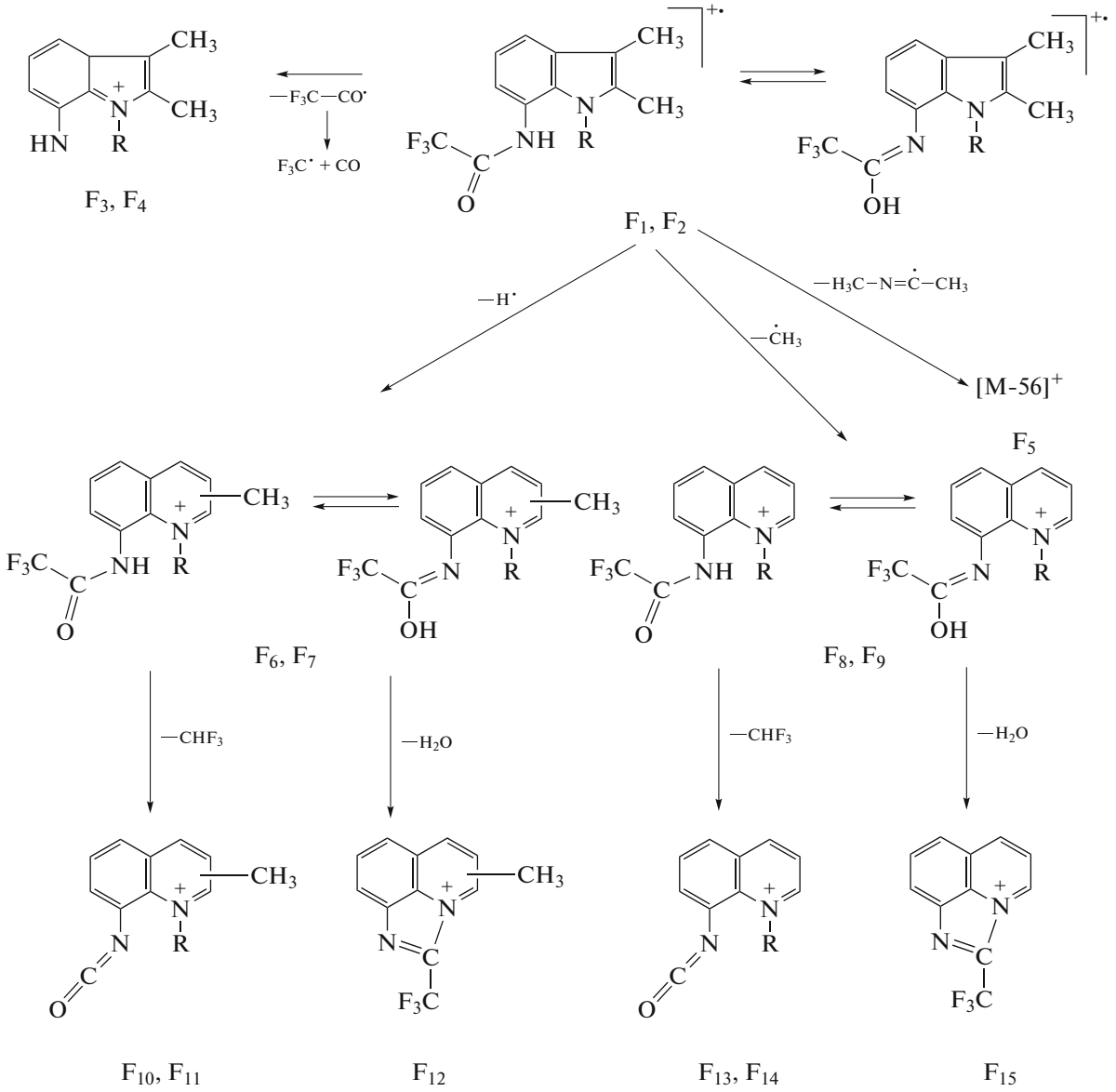
1, **2** **1**, **3** R = H; **2**, **4** R = CH_3 **3** (57%), **4** (65%)

Scheme 1.

According to the data of the quantum chemical calculations of the charges in the atomic charge units (a.c.u.) carried out by the density functional method (DFT) using the B3LYP hybrid functional and the 6–31G basis set on the nitrogen atoms of the amino group in aminoindoles **1** and **2** (–0.797 a.c.u. for compound 1 and –0.799 a.c.u. for compound **2**), their behavior in the trifluoroacetylation reaction was

expected to be the same. However, the formation of amide **4** is more difficult than compound **3** under the same conditions, which results in a longer reaction time (20–30 and 40–50 h for compounds **3** and **4**, respectively). This is probably due to the spatial difficulties created by the *N*-methyl group of the indole pyrrole ring. The chromatographic control of the reaction revealed two forms of compounds **3** and **4**: carbonyl (**a**) and enolic (**b**) with different R_f values.

In contrast to the UV spectra of the starting compounds **1** and **2** containing two absorption maxima (227, 275, and 232, 285 nm for amines **1** and **2**, respectively), three absorption bands appeared in the UV spectra of compounds **3** and **4** in ethanol with λ_{max} values of 212, 230, 298 nm for amide **3** and $212_{sh.}$ (shoulder), 232, 290 nm for amide **4**. The appearance of an additional band or shoulder (212 nm) is explained by the presence of an amide group in the molecule, while the shift of the maximum of the π–π transitions to the long-wavelength region is due to the elongation of the conjugation chain in the amide molecule.



R = H (F_1, F_3, F_6, F_8, F_{10}, F_{12}, F_{13}, F_{15}); R = CH_3 (F_2, F_4, F_5, F_7, F_9, F_{11}, F_{14})

Scheme 2.

Table 1. MIC values (μ/mL for compounds **3** and **4** and dioxidine for test strains of microorganisms

Test strain of the studied microorganism	Compound, MIC (μ/mL)		
	3	**4**	Dioxidine [11]
S. aureus 29213	31.3	62.3	125.0–1000.0
S. pyogenes 1238	31.3	31.3	–
E. coli 25922	125.0	125.0	8.0–250.0
P. aeruginosa 27853	125.0	125.0	125.0–1000.0
K. pneumoniae 9172	More than 250	125.0	8.0–250.0

The structure of compounds **3** and **4** was also confirmed by the data of the ^{1}H NMR spectra. A significant difference in the ^{1}H NMR spectra in the DMSO-d6 of the obtained amides **3**, **4** and aminoindoles **1**, **2** consisted in the absence of a signal from the protons of the NH_2 group in the 4.75 or 4.67 ppm region and in the presence of the N–H hydrogen atom signal with a chemical shift of 10.91 ppm (for **3a**), 11.31 ppm (for **4a**), as well as the proton of the O–H group with a chemical shift of 7.36 ppm (for **4b**). The signals of the hydrogen atoms of the 2,3-CH_3 group appeared in the spectra as two strong-field singlets and the protons of the ABC system of the benzene ring were characterized by two doublets and a triplet. In the ^{1}H NMR spectra, singlet signals ^{1}H (for **3**) and 1-CH3 (for **4**) were also present.

The ^{1}H NMR spectral analysis revealed that a form a of compound **3** existed in the DMSO-d6 solution, and a mixture of tautomers b and a was found for the structure of compound **4** (in a 3 : 1 ratio, according to the integrated intensity of the characteristic proton signals). The presence of the methyl group at the pyrrole nitrogen atom contributed to an increase in the stability of the enolic form b of compound **4**.

Additional information on the structure of amides **3** and **4** was obtained by mass spectral analysis. Studied compounds were stable under electron ionization conditions that was illustrated by the presence of peaks of molecular ions with the maximum intensity. Indolylamides **3** and **4** under the conditions of the registration of mass spectra were in two tautomeric forms, carbonyl (**a**) and enolic (**b**). Initially, the molecular ions F_1, F_2 of each of these forms either eliminated the hydrogen atom or the methyl radical turning into ions F_6, F_7, F_8, and F_9 (Scheme 2) as is typical for polymethylindoles [8–10].

The third direction of molecular ion decomposition was the formation of F_3 and F_4 fragment ions due to the cleavage of the F_3CCO radical from the carbonyl (**a**) amide form. The formation of the trifluoroacetyl fragment was also confirmed by the presence of signals of ions with m/z equal to 69 and 28 in the spectrum corresponding to the final products of the F_3CCO decay. Further transformation of the products of the elimination of H and CH_3, i.e., the F_6, F_7, F_8, and F_9 ions, occurred in two directions with the formation of fragments of either F_{10}, F_{11}, F_{13}, F_{14}, or F_{12}, and F_{15}. Fragmentary ions F_{10}, F_{11}, F_{13}, and F_{14} are formed from F_6, F_7, F_8, and F_9 ions in form a as a result of the removal of the trifluoromethane molecule; and fragmentary ions F_{12}, F_{15} are formed from F_6 and F_8 in form b due to the loss of a water molecule.

A significant difference in the mass-spectral decomposition of amides **3** and **4** was the absence of fragment ions resulting from F_7 and F_9 as a result of the splitting off of the water molecule. This confirmed that the formation of H_2O in the case of amide **3** involves the OH group and the hydrogen atom bound to the nitrogen atom of the pyrrole fragment of the enolic form b.

Another difference in the behavior of amides **3** and **4** under the electron ionization conditions was found: an intense (24.52%) signal of the F5 fragment ion (m/z = 214) was observed in the mass spectrum of compound **4**, the formation of which was presumably due to the elimination of the $[H_3C–N=C–CH_3]^{\bullet}$ radical. Such fragmentation of the pyrrole ring of the polymethylated indole system had not been previously described.

The screened indolylamides **3** and **4** were found to have antimicrobial activity. The research results in the form of the values of the minimum inhibitory concentrations (MICs) are shown in Table 1.

As shown in Table 1, the MICs of compound **3** for the studied gram-positive strains *S. aureus* 29213 and *S. pyogenes* 1238 was 31.3 μg/mL. Amide **3** was also effective against gram-negative strains. The growth of *E. coli* 25922 and *P. aeruginosa* 27853 was inhibited in the presence of 125 μg/mL of compound **3**, while for the *K. pneumoniae* the 9172 MIC value was more than 250 μg/mL. Compound **4** had the greatest antimicrobial activity against gram-positive test strains as amide **3** and the growth of *S. pyogenes* 1238 was inhibited in the presence of 31.3 μg/mL in the Mueller–Hinton broth (MHB). However, for *S. aureus* 29213, the MIC value of the studied compound **4** was slightly higher and amounted to 62.3 μg/mL. Compound **4** demonstrated a similar activity against gram-negative strains. For *E. coli* 25922 and *P. aeruginosa* 27853, its MIC value was 125 μg/mL, but in contrast to amide **3**, compound **4**

at a concentration of 125 μg/mL actively suppressed the growth of the *K. pneumoniae* 9172 strain. The detected antimicrobial activity of the studied compounds is not only inferior but exceeds the similar activity of the reference drug, dioxidine, which is able to suppress the growth of gram-positive strains of the *Staphylococcus* genera (in the range of 125 to 1000 μg/mL) and *Streptococcus* (in the range of 64 to 1000 μg/mL). In terms of the antimicrobial activity against gram-negative test strains, the compounds studied are also not inferior to the reference drug (Table 1).

EXPERIMENTAL

NMR 1H spectra were recorded using a Joel JNM-ECX400 multi-core nuclear magnetic resonance spectrometer (400 MHz) in DMSO-d6. Electronic spectra were obtained using LEKI SS2109UV instrument in ethanol. Mass spectra were recorded on a Finnigan MAT INCOS-50 mass spectrometer with direct sample injection into an ion source with an ionization energy of 70 eV. Elemental analysis was performed using a Vario MICRO cube elemental analyzer. Amines and amides are designated according to the rules of the ACD/LABS IUPAC Name Generator software. Structural formulas of the compounds were drawn in the ISIS Draw 2.4 software. To calculate the effective charges on the nitrogen atoms (in a.c.u.) in amines 1 and 2, the density functional method with the B3LYP hybrid functional [12] and the 6-31G basis set [13] was used, the calculations were carried out using the Orca software package [14].

The reaction products were purified by the method of column chromatography. Aluminum oxide was used as a sorbent (neutral, I and II according to the Brockmann activity). The control of the reactions, the purity of the compounds obtained, and the determination of R_f were carried out using TLC on Silufol UV-254 plates (the system was indicated specifically for each compound in the experimental procedure).

***N*-(2,3-Dimethyl-1*H*-indol-7-yl)-2,2,2-trifluoroacetamide (3).** Five mL (42 mmol) of trifluoroacetic acid ethyl ester and catalytic amount of glacial acetic acid was added to a solution of 0.32 g (2 mmol) of 7-amino-2,3-dimethylindole (1) in 150 mL of absolute benzene, and the mixture was boiled until the starting amine disappeared (chromatographic control). At the end of the reaction (30 h of boiling), benzene, the excess of ether, and traces of acetic acid were distilled off from the reaction mixture. The solid residue was dissolved in chloroform and passed through a layer of aluminum oxide. The yield of indolyltrifluoroacetamide was 57%, $T_{melt.}$ = 119–121°C, and R_f = 0.12; (benzene : ethyl acetate 5 : 1) 0.83. The following information was revealed by mass-spectrometry, %: C 56.03; H 4.19; and M 256. $C_{12}H_{11}F_3N_2O$. The following information was calculated, %: C 56.25; H 4.33; M 256. UV spectrum (ethanol) λ_{max} (log ε): 212 (4.47), 231 (4.37), and 298 (3.95) nm; ^{1}H NMR spectrum (DMSO-d_6): 2.12 (3H, c, 3-CH_3), 2.30 (3H, c, 3-CH_3), 6.92 (1H, t, J =7.8 Hz, H-5), 7.00 (1H, d, J = 7.8 Hz, H-6), 7.27 (1H, d, J = 7.8 Hz, H-4), 10.57 (1H, c, H-1), and 10.91 (1H, c, H–*N*) ppm. Mass spectrum *m/z* (% of J_{max}): 256 (100), 255 (34.13), 241 (10.61), 237 (15.82), 223 (10.81), 185 (5.61), 159 (7.71), 69 (7.11), 28 (7.41), and 18 (17.22).

***N*-(1,2,3-Trimethyl-1*H*-indol-7-yl)-2,2,2-trifluoroacetamide (4).** Compound **4** was obtained similarly from 0.35 g (2 mmol) of 7-amino-1,2,3-trimethylindole (2); however, the reaction mixture was heated for 50 h to complete the reaction. The obtained amide was purified on a column with aluminum oxide in the system chloroform: petroleum ether = 5 : 1. The yield was 65%, $T_{melt.}$ = 126–128°C, and R_f = 0.21; 0.87 (benzene: ethyl acetate = 5 : 1). The following information was revealed by mass-spectrometry, %: C 57.32; H 4.70; M 270. $C_{13}H_{13}F_3N_2O$. The following information was calculated, %: C 57.48, H 4.85; and M 270. UV spectrum (ethanol) λ_{max} (log ε): 212 (4.40), 232 (4.73), 290 (4.15) nm, ^{1}H NMR spectrum (DMSO-d_6) tautomeric form **a**: 2.18 (3H, c, 3-CH_3), 2.30 (3H, c, 3-CH_3), 3.69 (3H, c, 1-CH_3), 6.88 (1H, d, J = 8.0 Hz, H-6), 6.99 (1H, t, J = 8.0 Hz, H-5), 7.42 (1H, d, J = 8.0 Hz, H-4), 11.35 (1H, C, H–*N*) ppm; tautomeric form **b**: 2.18 (3H, c, 3-CH_3), 2.31 (3H, c, 2-CH_3), 3.94 (3H, c, 1-CH_3), 6.99 (1H, t, J = 8.0 Hz, H-5), 7.17 (1H, d, J = 8.0 Hz, H-6), 7.36 (1H, c, H–*O*), 7.42 (1H, d, J = 8.0 Hz, H-4) ppm. The ratio between the tautomeric form a and tautomeric form b, according to the integrated intensity of the characteristic proton signals in the ^{1}H NMR spectrum, was 1 : 3. Mass spectrum *m/z* (% of J_{max}): 270 (100), 269 (18.62), 255 (16.52), 239 (6.71), 214 (24.52), 201 (8.00), 199 (15.02), 185 (6.51), 184 (10.31), 183 (8.81), 173 (43.74), 69 (10.51), and 28 (10.71).

Antibacterial activity of *N*-(2,3-dimethyl-1*H*-indol-7-yl) and *N*-(1,2,3-trimethyl-1*H*-indole-7-yl)-2,2,2-trifluoroacetamides (3, 4). For microbiological experiments, the studied compounds were used in the form of a solution. Dimexide was used as a solvent for preparing solutions for external use (OAO Marbiopharm). Five museum strains *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Streptococcus pyogenes* ATC 1238, and *Klebsiella pneumoniae* ATCC 9172 were used as test microorganisms for the determination of the antimicrobial activity of compounds **3** and **4**. The strains used in the work were obtained from the collection of the Museum of Living Cultures FSBI SCEEMP of the Ministry of Health of the Russian Federation. The antimicrobial activity of amides **3** and **4** was determined by the serial dilution method in the Mueller–Hinton broth (tube macro method) (according to MUK 4.2.1980-04) [15]. An antimicrobial drug dioxidine (a derivative of di-*N*-hydroxyquinoxaline)

with a high level of chemotherapeutic activity, which is widely used in medical practice, was used as the reference drug.

COMPLIANCE WITH ETHICAL STANDARDS

This article does not contain any research using animals as objects.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Yamashkin, S.A., Pozdnyakova, O.V., and Yurovskaya, M.A., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2014, vol. 69, no. 1, p. 31.
2. Yamashkin, S.A., Zhukova, N.V., and Romanova, I.S., *Chem. Heterocycl. Compd.*, 2008, vol. 44, no. 7, p. 793.
3. Kadimaliev, D.A., Stepanenko, I.S., Nadezhina, O.S., and Yamashkin, S.A., *Mikol. Fitopatol.*, 2014, vol. 48, no. 5, p. 309.
4. Stepanenko, I.S., Kot'kin, A.I., and Yamashkin, S.A., *Probl. Med. Mikol.*, 2015, vol. 17, no. 3, p. 135.
5. Kay, C.W.M., Mennenga, B., Görisch, H., and Bittl, R., *J. Biol. Chem.*, 2006, vol. 281, no. 3, p. 1470.
6. Stepanenko, I.S., Kot'kin, A.I., and Yamashkin, S.A., *Fundam. Issled.: Khim. Nauki*, 2013, no. 8, p. 1406.
7. Alyamkina, E.A., Stepanenko, I.S., Yamashkin, S.A., and Yurovskaya, M.A., *Moscow Univ. Chem. Bull. (Engl. Transl.)*, 2017, vol. 72, no. 1, p. 24.
8. Khmel'nitskii, R.A., *Chem. Heterocycl. Compd.*, 1974, vol. 10, no. 3, p. 253.
9. Terent'ev, P.B., *Mass-spektrometriya v organicheskoi khimii* (Mass Spectrometry in Organic Chemistry), Moscow: Vysshaya Shkola, 1979.
10. Lebedev, A.T., *Mass-spektrometriya v organicheskoi khimii* (Mass Spectrometry in Organic Chemistry), Moscow: BINOM, 2010.
11. Padeiskaya, E.N., *Infekts. Antimikrob. Ter.*, 2001, vol. 3, no. 5, p. 105.
12. Stephens, P.J., Devlin, F.J., Chablowski, C.F., and Frisch, M.J., *J. Phys. Chem.*, 1994, vol. 98, p. 11623.
13. Hehre, W.J., Ditchfield, R., and Pople, J.A., *J. Chem. Phys.*, 1972, vol. 56, p. 2257.
14. Neese, F., *Mol. Sci.*, 2012, vol. 2, no. 1, p. 73.
15. MUK (Methodological Guideline) 4.2.1890-04: Determination of the sensitivity of microorganisms to antibacterial preparations, *Klin. Mikrobiol. Antimikrob. Khimioter.*, 2004, vol. 6, no. 4.

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