THE SYNTHESIS AND COUPLING WITH MAGNETITE NANOPARTICLES OF 4,6-DIMETHYL-2-(2-OXYETHYL)-1,2-DIHYDRO-3H-PYRROLO[3,4-C] PYRIDINE-3-ONE AND CHARACTERIZATION ITS STRUCTURE

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In this paper is reported the synthesis of new derivative of pyrrolo-pyridine condensed heterocycle that might reveal biological activity, similar to nootropic effects. The 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one was synthesized by reaction of ethyl 2,6-dimethyl-4-(chloromethyl)pyridine-3-carboxylic acid with ethanolamine. NMR, IR spectroscopy and elemental analysis methods characterized the structure of synthesized compound. Further, via self-assembling of synthesized heterocyclic compound on the surface of magnetite nanoparticles, were obtained the nanostructures. The morphology of prepared nanostructures was studied by Scanning Electron Microscopy (SEM), and it was revealed that the prepared nanoparticles (NPs) were homogeneous in size and have spherical shape. Quantitative analysis of coupled heterocyclic molecules was performed by atom absorbance spectroscopy. The samples also were analyzed by Fourier Transform Infrared (FTIR) and powder X-ray diffraction (XRD) spectroscopy.

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1. Introduction

The derivatives of pyridine heterocycle are well-known for their effect on central nervous system. The vitamin B6 (pyridoxine) and niacin have both the pyridine ring in their structure, which is responsible in their action on nerve cell metabolism. [1] The racetams, derivatives of pyrrolidone, are recognized and are widely used nootropic, stimulant and anticonvulsant drugs. [2] Pyrazolo-pyridine moiety and different types of their derivatives show selectivity to D4 receptors, and can be applied in diagnosis of Parkinson's disease. [3] Also, pyridine derivatives are seemed to be a promising drug for the treatment of Alzheimer's disease. [4] At the same time, the nano-scaled material, due to the enormous increase of its surface area to volume ratio, acquires unique properties. This fundamental concept is valid in term of creation of technologies of nanoscale targeted drug delivery systems. Nano-formulations comparing with traditional drugs have advantages, due to penetration into target cells, while leaving normal cells intact [5]. In addition, the engineering of nanotechnology-based drug delivery systems have a significant impact on the optimization of dosage, stability and effectiveness of the drug. [6,7] Along with the above benefits nanosystems can deliver the drug in a well-defined period of time. It is obvious that based on magnetite nanoparticles targeting drug delivery occurs, due to high superparamagnetic characteristics [8,9] of these nanoparticles.

In the paper we report of synthesis of new derivative of pyrrolo-pyridine condensed heterocycle (HC), having both pyridine and pyrrolidone nucleus in its structure with further nano-coupling with Fe_3O_4 nanoparticles and characterization of their structure.

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2. Materials and methods

All chemicals, used in the synthesis, were of analytical grade and used as received. Ethyl ether of 2,6-dimethyl-4-(chloromethyl)pyridine-3-carboxylic acid (1), ethanol, ethanolamine were purified by distillation under reduced pressure, created by water pump. K_2CO_3, FeCl3·6H2O, FeSO4·7H2O, NH4OH (25 %), were purchased from Sigma-Aldrich (Taufkirchen, Germany)

Synthesis of 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one (HC)

To a solution of 20mmol of a 3-pyridine carboxylic acid (1) in 20ml of ethanol successively with stirring dropwise at 25-30 ° C were added 20mmol of ethanolamine, then 1.38g (10mmol) of K_2CO_3, dissolved in 50 ml of water. The reaction mixture was stirred at 70-75 °C for 5h. After cooling 100 ml of water was added; the precipitated crystals were filtered, washed with cold water and recrystallized from water. 78% yield, colorless crystals, m.p. 133-134 °C. IR spectrum in cm^{-1}; 3035 (= CH), 1670 (lactam C = O), 1560.1505 (C = C). The NMR experiments have been performed on a BRUKER FT NMR spectrometer AVANCE 300 (Bruker, Karlsruhe, Germany) (300 MHz for ^1H and 75 MHz for ^{13}C) with a BVT 3200 variable temperature unit in 5 mm sample tubes, using Bruker Standard software (TopSpin 3.1). The ^1H and ^{13}C chemical shifts were referenced to internal tetramethylsilane (TMS); the experimental parameters for ^1H are as follows: digital resolution = 0.23 Hz, SWH = 7530 Hz, TD = 32 K, SI = 16 K, 90^0 pulse-length = 10 μs, PL1 = 3 dB, ns=1, ds=0, d1=1 s and for ^{13}C as follows: digital resolution = 0.27 Hz, SWH = 17985 Hz, TD = 64 K, SI = 32 K, 90^0 pulse-length = 9 μs, PL1 = 1.5 dB, ns=100, ds=2, d1=3 s.

^1H NMR spectra of 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one: (DMSO-d_6, δ, ppm), 2.52 s (3H, CH_3), 2.71 s (3H, CH_3), 3.55 t (2H, CH_2), 3.62 q (2H, CH_2), 4.45 s (2H, CH_2), 4.68 t (1H, OH), 7.14 s (1H, Ar).

^{13}C NMR spectra of 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one (HC): (DMSO-d_6, δ, ppm), 20.5 (CH_3), 24.8 (CH_3), 45.4 (CH_2), 50.8 (CH_2), 60.5 (CH_2), 115.7 (CH, Ar), 123.6 (C, Ar), 152.1(C, Ar), 155.7 (C, Ar), 159.7 (C, Ar), 168 (CO).

Synthesis of nanostructures by coupling of 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one (HC) with Fe_3O_4 nanoparticles: HC@Fe_3O_4

Magnetic iron oxide nanoparticles are usually prepared by wet chemical precipitation from aqueous iron (II) and iron (III) salt solutions in alkaline media, created by liquid ammonia, in the atmosphere of gaseous nitrogen, as described in literature [10]. The formed magnetite nanoparticles (NPs) were separated by strong NdFeB permanent magnet, repeatedly washed with distilled water and dispersed in ethanol. The ethanol solution of HC, taken in excess, was added to ethanol solution of Fe_3O_4 nanoparticles and vigorously stirred 3 hours. Then nanoparticles were coupled with 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one molecules, by adding the ethanol solution of HC into reaction mixture by means of self-assembling. After stirring during 5 hours at ambient, the prepared nanostructures were separated by strong NdFeB permanent magnet and repeatedly were washed with distilled water. The obtained NPs of HC@Fe_3O_4 were dried at ambient conditions, and the iron content in the samples was analyzed by atom absorption spectroscopy and performed on Varian SpectrAA 220FS Atomic absorption spectrometer. Samples were prepared by Milestone ETHOS 1 Microwave extraction unit.

Characterization of structure

Powder X-ray diffraction (XRD)

X-ray diffraction analysis was performed on Rigaku Mini Flex 600 XRD diffractometer at ambient. In all the cases, Cu K radiation from a Cu X-ray tube (run at 15 mA and 30 kV) was used. The samples were scanned in the Bragg angle 2h range of 20–70 °.

Fourier Transform Infrared spectroscopy (FT-IR)

The functional groups, present in the powder samples of HC@Fe_3O_4, were identified by Fourier Transform Infrared (FTIR) spectroscopy. FTIR spectra were recorded on a Varian 3600

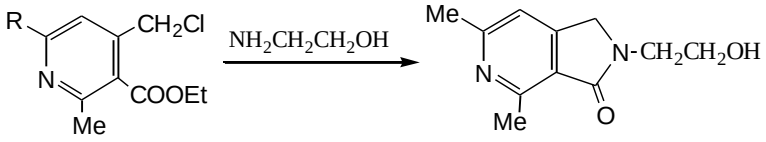
FTIR spectrophotometer in KBr tablets. The spectrum was taken in the range of 4000-400 cm^{-1} at room temperature.

Scanning Electron Microscope (SEM) and Energy-Dispersive Spectrum (EDS) analysis

SEM and EDS analysis of prepared samples of HC@Fe_3O_4 nanoparticles were taken on Field Emission Scanning Electron Microscope JEOL JSM-7600F at an accelerating voltage of 15.0 kV, SEI regime.

3. Results and discussion

Considering that heterocycles, having in their structure pyridinium and pyrollidone moieties, reveal a broad spectrum of biological activity [11,12], it seemed perspective to us to synthesize a novel pyrrolo-pyridine fused heterocycle (HC). 2,3-dihydro-pyrrolo [3,4-c] pyridin-1-ones exhibit analgesic and psychotropic [13] and tetrahydropyrrolo [3,4-c] pyridine-4-ones are effective for the treatment of obesity and neurological disorders [14]. Reaction of ethyl ether of 2,6-dimethyl-4- (chloromethyl) pyridine-3-carboxylic acid with monoethanolamine led to synthesis of 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one. Derivatives of pyridine-3-carboxylic acid (1) are a promising synthon for heterocyclization them into pyrrolo [3,4-c] pyridines. Based on them, depending on the primary amine, can be synthesized previously unknown pyrrolo [3,4-c] pyridines, having various functional groups that reveal pharmacophoric features.



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Scheme 1. Reaction of synthesis of 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one (HC)

Further coupling of synthesized HC with magnetite nanoparticles was carried via self-assembling by means of non-covalent bonds, forming between the surface of Fe_3O_4 nanoparticles and molecules of HC.

The purity and crystalline properties of the HC@Fe_3O_4 were investigated by powder X-ray diffraction (XRD). All the XRD peaks, shown on figure 1, were well defined and corresponded to Fe_3O_4 nanoparticles with cubic structure. In XRD peak broadening testifies for the formation of nanocrystals with amorphous degree of crystallinity. In the pattern all lines relate to magnetite and can be indexed, using the ICDD (PDF-2/Release 2011 RDB) DB card number 00-001-1111, having characteristic peaks at 35.73° (311), 37.37°(222), 43.43° (400), 53.89° (422), 57.45°(511), 63.09° (440), which correlate with the standard pattern of Fe_3O_4 well. The intensity of the diffraction peak of (311) is stronger than the other peaks. The average crystal size, estimated from (311) peak, using the Scherrer formula is 11.5 nm.

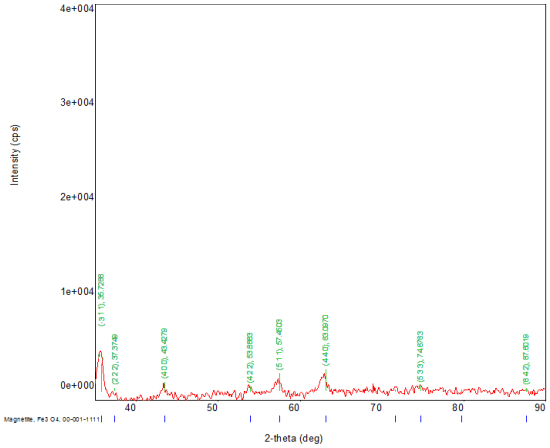

Fig. 1. XRD pattern of HC@Fe_3O_4 nanostructures

On the Fig. 2 is shown the IR spectra of nanostructured HC@Fe_3O_4 (a) and pure HC (b). On the spectra of pure HC (b) are bands of different intensities in the region of 1616–1074 cm^{-1}; where peaks within the ranges 1616–1319 are corresponding to stretching vibrations of C–C and C–N in pyridine ring. In the region of 1224–943 cm^{-1} there are bands, corresponding to deformation vibrations of CH groups in and beyond the plane of pyridine cycle. The intense band at 1670 cm^{-1} corresponds to stretching vibrations of C=O group of δ-lactam, and broad bands, which are at 3435 and 3293 cm^{-1}, are characteristic to inter and intramolecular bonded OH that also revealed medium peak at 1430-1320 cm^{-1}, corresponding to bending vibration of alcohol group. The spectrum of nanostructures HC@Fe_3O_4 (a) exhibits a characteristic peak of Fe_3O_4 at about 572-585 cm^{-1}, corresponding to Fe–O stretching, testifying the coupling of HC molecules with magnetite nanoparticles. The disappearance of bands, which are attributed to OH group at 3435, 3293 cm^{-1} in the IR spectrum (a), demonstrates that chelating of HC with the surface of magnetite nanoparticles occurs via hydroxyl group of HC molecules. The shift of intense band at 1670 cm^{-1} of stretching vibrations of C=O group of δ-lactam (b) to 1700 cm^{-1} region (a) indicates that this moiety is also involved into coordination process with Fe_3O_4 nanoparticles. The comparative analysis of both spectra of pure HC (b) and nanostructures HC@Fe_3O_4 (a) revealed multiple chelation sites of HC via non-covalent interactions with the surface of magnetic NPs.

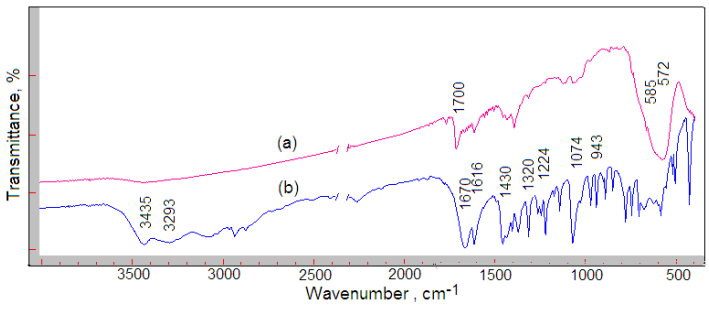

Fig. 2. The IR spectra of nanostructured HC@Fe_3O_4 (a) and pure HC (b)

The surface morphology of the HC@Fe_3O_4 was determined by SEM and is shown in Figure 3. The points, identified in the EDS spectra in Fig. 4, demonstrate the presence of Fe and O as the main elements of the sample and support the data of magnetite nanoparticles formation (the other peaks are being characteristic of the carbon-coated grid).

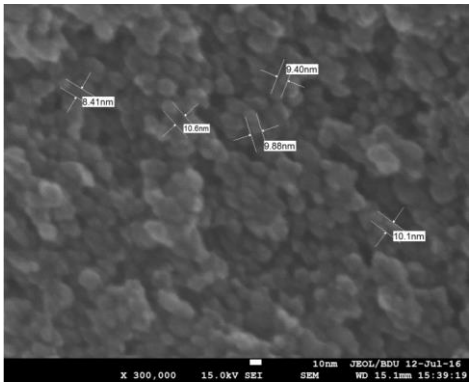

Fig. 3. SEM image of HC@Fe_3O_4

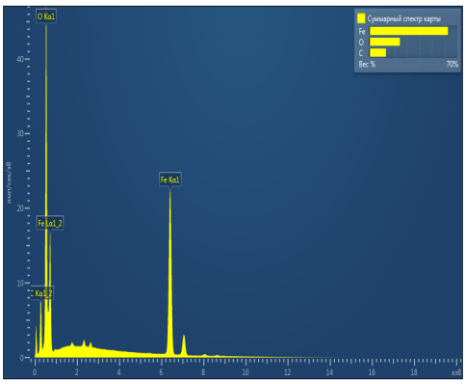

Fig. 4. ED pattern of HC@Fe_3O_4

As it is shown in Figure 3, the prepared nanostructures are monodisperse with almost uniform size approximately 8-10.5 nm. The average sizes of formed nanoparticles correlate well with the data, obtained from XRD analysis.

Quantitative analysis of HC@Fe_3O_4 nanostructures was performed by AAS method, by determination of the iron content and further calculation of HC quantity in the samples. It was found that 1 g of HC@Fe_3O_4 nanostructures contains 0.23 g of HC.

4. Conclusions

In recent years much attention is paid to the construction of new nanocomposites that can be useful in neurodegenerative diseases [15, 16, 17]. Since heterocyclic compounds, containing pyridine and pyrrol fragments in its structure, often exhibit biological activity towards the treatment of these diseases, design and modification of their derivatives are extensively investigated. The application of nanoparticles, especially magnetite, combined with bioactive compound, can facilitate the penetration of the drugs into brain cells and perform the action in lower dosage, due to the avoiding of medicine's leakage [18]. We report the synthesis of new derivative of pyrrolo-pyridine condensed heterocycle, offering the biological activity, similar to neurotropic effects. The 4,6-dimethyl-2-(2-oxyethyl)-1,2-dihydro-3H-pyrrolo[3,4-C]pyridine-3-one was synthesized by reaction of ethyl 2,6-dimethyl-4-(chloromethyl)pyridine-3-carboxylic acid with ethanolamine in good yield. The further coupling of biologically active compounds with biocompatible Fe_3O_4 nanoparticles offers great opportunities to modify the properties of these compounds at the molecular level and might show unprecedented biological performance.

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