SYNTHESIS, DRUGLIKENESS ESTIMATION AND PREDICTION OF POSSIBLE PHARMACOLOGICAL EFFECTS OF NEW PYRROLE HYDRAZONES

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ABSTRACT

The synthesis of five new N-pyrrolyl derivatives is presented. A classical Paal-Knorr cyclization was used for synthesis of the initial N-pyrrolyl hydrazide and the final hydrazones were obtained in a micro synthesis apparatus, assuring about 65 – 95% yields, low harmful emissions and reagent economy. The compounds were elucidated by IR and ¹H NMR spectral analyses and the obtained results were consistent with the assigned structures. The purity of the substances was proven by TLC characteristics and corresponding melting points. In addition, an attempt to predict the pharmacokinetic behavior and possible pharmacological effects of the target compounds was made, based on the molecular descriptors, obtained by the Molinspiration Cheminformatics web-based tool.

Keywords: pyrrole hydrazones, synthesis, pharmacokinetics

INTRODUCTION

Pyrrole is widely known as a biologically active scaffold which possesses a diverse spectrum of activities. The combination of different pharmacophores in a pyrrole ring system has led to the formation of more active compounds. Pyrrole-containing analogs are considered as a potential source of biologically active compounds containing a significant set of advantageous properties and can be found in many natural products (1).

Pyrrole is a five-membered heterocyclic compound, corresponding to the C₄H₄NH general formula (2). It is a colorless volatile liquid, unstable in the presence of air, where it easily darkens (3).

Pyrrole cannot be found in nature by itself, but a lot of its derivatives are components in the structure of a number of co-factors, natural products and marketed drugs. It is a major structural component in the structure of more complex compounds like vitamin B12, bilirubin, heme b, chlorophyll, etc. (4,5).

Pyrrole and its derivatives play an important role in pharmaceutical and medicinal chemistry. Commonly they are widely used as an intermediate in the synthesis of new molecules applied as drugs, pharmaceuticals and other organic compounds like chlorophyll and heme-derivatives made by four pyrrole ring formation of porphyrin ring system.

On the other hand, hydrazide-hydrazone group is found in a number of bioactive compounds, expressing a variety of pharmacological effects like antibacterial, antitubercular, antifungal, anti-inflam-

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Figure 1. Heme b

matory, anticonvulsant, antiviral and antiprotozoal ones (6).

The identification of lead compounds showing pharmacological activity against specific targets and the progressive optimization of the pharmacological properties and potency of these compounds are the focal points of early-stage drug discovery. The structure-based drug design and ligand-based analysis is a helpful tool for fast and cost-effective structural optimization and identification of lead molecules. The knowledge on the three-dimensional (3D) structure of the designed compounds and biological targets in the process is a premise to obtaining highly effective and potent new pharmacologically active molecules (7).

AIM

The aim of our study is to evaluate the possible pharmacological effect of newly synthesized in the study N-pyrrolyl hydrazide-hydrazones.

MATERIALS AND METHODS

General

The final hydrazones were synthesized in an apparatus for micro-scale synthesis (ALDRICH Ace Micro-LabKit, St. Louis, USA) assuring low harmful emissions and reagent economy. The melting points were determined with a capillary Digital Melting

Point Apparatus IA 9200 ELECTROTHERMAL (Southend-on-Sea, England) and were not corrected. The synthesis progress was controlled by TLC on aluminium sheets Silica gel 60 F₂₅₄ (Merck, Darmstadt, Germany), using CHCl₃/CH₃CH₂OH as a mobile phase. Yields were calculated for purified products.

The IR spectra were taken on SPECORD 71 IR (Carl-Zeiss, Jena, Germany). The ¹H NMR spectra were registered at 250 MHz on spectrometer Bruker-Spectrospin WM250MHz (Faenland, Switzerland) as δ (ppm) relative to TMS as internal standard with HDMSO as a standard (20°C); coupling constants (*J*) are expressed in Hertz (Hz). All OH and NH protons were D₂O exchangeable.

The molecular descriptors and possible pharmacological effects were determined using the free web based server Molinspiration Cheminformatics (8-10).

All commercial chemicals and reagents used as starting materials in this study were purchased from Merck (Darmstadt, Germany).

Synthesis of Carbohydrazide III

Since carbohydrazide **III** was not found to be reported in the literature, it was prepared by selective hydrazinolysis of the relevant intermediate ethyl ester according to the published general procedure for synthesis of N-pyrrolyl carbohydrazides (11) and given in **Figure 3**.

The IUPAC name¹ of the newly synthesized hydrazide, together with the interpretation of its IR and ¹H NMR spectra are presented below:

Ethyl 5-(4-chlorophenyl)-1-(1-hydrazinyl-1-oxo-3-phenylpropan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (**III**): IR in KBr, [cm⁻¹]: 3200 (NH), 1690 (COOC₂H₅), 1640 (Amide I), 1570 (Amide II), 1240 (C-O), 820 (p-disubstituted C₆H₄); ¹H NMR (at 250 MHz, in CDCl₃): 1.30 (t, *J*=7.1Hz, 3H, CH₃CH₂), 2.50 [s, 3H, CH₃(2)], 3.20-3.35 (m, 2H, CH₂-CH), 3.60 (brs, 2H, NH₂), 4.20 (q, *J*=7.1Hz, 2H, OCH₂CH₃), 4.75-4.85 (m, 1H, CH₂-CH), 6.20 [s, 1H, H(4)], 6.35-6.60 (m, 5H, C₆H₅), 6.80-7.15 (m, 4H, C₆H₄), 7.90 (m, 1H, NH);

General Procedure for the Synthesis of the Targeted Hydrazones:

First, 1.5 mmol of carbohydrazide **III** and 1.5 mmol of any of the carbonyl partners **a**, **b**, **c** or **d** were dissolved in 4.5 ml glacial acetic acid in a reaction

1. ACD/Name Version 2.51

vial of 5 ml. The mixture was stirred at 100 °C for 40-50 min to complete the reaction under TLC-control. The products were isolated after adding water and recrystallized from ethanol.

The IUPAC names¹ of the newly synthesized hydrazones together with the interpretation of their IR and ¹H NMR spectra are presented below:

(E)-ethyl-1-(2-(2-benzylidenehydrazinyl)-1-oxo-3-phenylpropan-2-yl)-5-(4-chlorophenyl)-2-methyl-1H-pyrrole-3-carboxylate (**IIIa**): IR in KBr, [cm⁻¹]: 3200 (NH), 1690 (COOC₂H₅), 1620 (Amide I), 1520 (Amide II), 1240 (C-O), 820 (p-disubstituted C₆H₄), 750, 700 (C₆H₅); ¹H NMR (at 250 MHz, in CDCl₃): 1.25 (t, J=7.1Hz, 3H, CH₃CH₂), 2.16[s, 3H, CH₃(2)], 3.10-3.25 (m, 2H, CH₂-CH), 4.30 (q, J=7.1Hz, 2H, OCH₂CH₃), 4.85- 4.95 (m, 1H, CH₂-CH), 6.25[s, 1H, H(4)], 6.85-7.00 (m, 5H, C₆H₅CH₂), 7.10-7.25 (m, 4H, C₆H₄), 7.60-7.80 (m, 5H, C₆H₅CH), 8.10 (s, 1H, CH=N), 8.40 (s, 1H, NH);

(E)-ethyl-5-(4-chlorophenyl)-1-(1-(2-(2-hydroxybenzylidene)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (**IIIb**): IR in KBr, [cm⁻¹]: 3120 (OH and NH), 1695 (COOC₂H₅), 1640 (Amide I), 1520 (Amide II), 1240 (C-O), 810 (p-disubstituted C₆H₄), 760 (o-disubstituted C₆H₄); ¹H NMR (at 250 MHz, in CDCl₃): 1.35 (t,

J=7.1Hz, 3H, CH₃CH₂), 2.65[s, 3H, CH₃(2)], 3.50-3.80 (m, 2H, CH₂-CH), 4.40 (q, J=7.1Hz, 2H, OCH₂CH₃), 4.90-5.00 (m, 1H, CH₂-CH), 6.40[s, 1H, H(4)], 6.80-7.00 (m, 4H, C₆H₄OH), 7.20-7.45 (m, 4H, C₆H₄), 7.60-7.85 (m, 5H, C₆H₅), 8.40 (s, 1H, CH=N), 10.30 (s, 1H, NH), 11.20 (br s, 1H, OH);

(E)-ethyl-5-(4-chlorophenyl)-1-(1-(2-(furan-2-ylmethylene)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)-2-methyl-1H-pyrrole-3-carboxylate (**IIIc**): IR in KBr, [cm⁻¹]: 3200 (NH), 1690 (COOC₂H₅), 1620 (Amide I), 1520 (Amide II), 1240 (C-O), 820 (C₆H₄), 750, 700 (C₆H₅); ¹H NMR (at 250 MHz, in CDCl₃): 1.15 (t, J=7.1Hz, 3H, CH₃CH₂), 2.50[s, 3H, CH₃(2)], 3.30-3.45 (m, 2H, CH₂-CH), 4.30 (q, J=7.1Hz, 2H, OCH₂CH₃), 4.95-5.10 (m, 1H, CH₂-CH), 6.10[s, 1H, H(4)], 6.35 (m, 1H, CH=N), 7.10-7.25 (m, 5H, C₆H₅CH₂), 7.30-7.75 (m, 4H, C₆H₄), 7.80-7.90 (m, 3H, FurH), 8.55 (s, 1H, NH);

(E)-ethyl-5-(4-chlorophenyl)-2-methyl-1-(1-(2-(3-nitrobenzylidene)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)-1H-pyrrole-3-carboxylate (**IIId**): IR in KBr, [cm⁻¹]: 3200 (NH), 1650-1680 (COOC₂H₅, Amide I), 1540 (Amide II), 1360 (NO₂), 1250 (C-O), 820 (C₆H₄), 750, 700 (C₆H₅); ¹H NMR (at 250 MHz, in CDCl₃): 1.20 (t, J=7.1Hz, 3H, CH₃CH₂), 2.35[s, 3H, CH₃(2)], 3.20-3.35 (m, 2H, CH₂-CH), 4.40

Figure 2. Synthesis of the initial N-pyrrolyl carboxylic acids

I: X = Br; n = 0; R = H

II: X = Br; n = 1; R = H

III: X = Cl; n = 0; R = CH₂C₆H₅

Figure 3. Synthesis of the new N-pyrrolyl hydrazide

(q, $J=7.1\text{Hz}$, 2H, OCH_2CH_3), 4.90-5.00 (m, 1H, $\text{CH}_2\text{-CH}$), 6.40[s, 1H, H(4)], 6.90-7.10 (m, 5H, $\text{C}_6\text{H}_5\text{CH}_2$), 7.50-7.75 (m, 4H, C_6H_4), 7.80-7.90 (m, 4H, $\text{C}_6\text{H}_4\text{CH}$), 8.20 (s, 1H, CH=N), 10.10 (s, 1H, NH);

RESULTS AND DISCUSSION

The initial for the synthesis N-pyrrolyl carboxylic acids were synthesized according to a procedure explained elsewhere (12,13) via classical Paal-Knorr cyclization as presented on **Figure 2**.

Carbohydrazides **I** and **II** were obtained according to a published procedure (14, 15), while carbohydrazide **III** was not found to be reported in the literature and was prepared according to the procedure given in **Figure 3**.

The purity of the new compound was confirmed by TLC analysis and melting point, and its structure was elucidated with IR and ^1H NMR spectral data. The corresponding purity characteristics are presented in **Table 1**.

Table 1. Purity characteristics and yield of carbohydrazide **III**

Compound	TLC: Rf/ ($\text{CHCl}_3:\text{C}_2\text{H}_5\text{OH}$)	M.p. ($^\circ\text{C}$)	Yield (%)
III	0.37/ (10:0.5)	138-140	75

The target hydrazones were obtained according to the procedure defined on **Figure 4** through condensation of the corresponding N-pyrrolyl hydrazide with any of the carbonyl compounds **a – d** presented on **Figure 5**:

The target products were synthesized in micro synthesis conditions, ensuring about 65 – 85% yields, low harmful emissions and reagent economy. The purity of the new compounds was con-

Figure 5. Used carbonyl compounds

firmed by TLC analysis and their structure was elucidated, with IR and ^1H NMR spectral data. The corresponding melting points, TLC characteristics and yields are presented in **Table 2**.

Table 2. Melting points, TLC characteristics (Rf/ ($\text{CHCl}_3:\text{C}_2\text{H}_5\text{OH}$) and yields (%)

Compound	TLC: Rf/ ($\text{CHCl}_3:\text{C}_2\text{H}_5\text{OH}$)	M.p. ($^\circ\text{C}$)	Yield (%)
Ia	0.53/ (10:0.5)	227-228	78
Ib	0.76/ (10:0.5)	226-227	68
Ic	0.72/ (10:0.75)	269-270	84
IIa	0.41/ (10:0.25)	150-152	78
IIb	0.43/ (10:0.25)	210-211	68
IIc	0.59/ (10:0.5)	154-155	84
IIIa	0.47/ (10:0.5)	169-170	82
IIIb	0.58/ (10:0.5)	144-145	79
IIIc	0.51/ (10:0.5)	113-115	72
IIId	0.42/ (10:0.5)	108-110	74

The obtained structures, along with some molecular parameters are presented in **Table 3**.

Figure 4. General synthesis of the target compounds

Table 3. Structure and some molecular parameters of the new hydrazones

Compound	Molecular parameters					% ABS
	Log P	MW	n (O, N)	n (OH,NH)	TPSA	
Ia	4.83	468.35	6	1	72.70	83.9185
Ib	4.774	484.35	7	2	92.93	76.93915
Ic	4.09	458.31	7	1	85.84	79.3852
IIa	5.11	482.38	6	1	72.70	83.9185
IIb	5.045	498.377	7	2	92.93	76.93915
IIc	4.36	472.34	7	1	85.84	79.3852
IIIa	6.49	514.02	6	1	72.70	83.9185
IIIb	6.43	530.02	7	2	92.93	76.93915
IIIc	5.75	503.99	7	1	85.84	79.3852
IIId	6.43	559.02	9	1	118.53	68.10715

Druglikeness Estimation and In Silico Screening for Possible Pharmacological Effects

A good evaluation tool for the molecules behavior in the organism is the determination of the so-called **druglikeness** of a newly synthesized organic structure. This parameter is based on a complex comparison of the compound molecular properties of the new drug candidate with a group of known drugs. The compounds molecular properties and structure features like hydrophobicity, electronic distribution, hydrogen bonding characteristics, molecule size and flexibility are the main pharmacophoric features determining the molecules bioavailability, transport properties, affinity to proteins, reactivity, toxicity, metabolic stability and many others.

Druglikeness Estimation with Lipinski's Rule of Five

The favorable pharmacokinetic behavior of a molecule is of great importance for its consideration as a potential drug. The possibility to ensure the needed bioavailability, transportation to the target through a number of membranes and the optimal metabolism and elimination of the substance are important parameters in the development of potential new drugs.

The reliability of the evaluated N-pyrrolyl hydrazide-hydrazones as possible prototypes in future structure property optimizations was assessed by Lipinski's Rule of Five (8) determining the following boundaries for some molecular parameters: hydrophobic parameter Log P<5, molecular weight MW<500, number of hydrogen bond acceptors n(O,

N) <10 and number of hydrogen bond donors n(OH, NH) <5. Thus, the compliance with these limits will be a premise to decreased risk of pharmacokinetic concerns.

Taking into account the importance of Lipinski's Rule of Five and the possibility for its application as a preselection tool, Lipinski's parameters were calculated, using the Molinspiration Cheminformatics web server and the obtained results are presented in **Table 3**. In addition, using the proposed formula (1) the corresponding Percentage Absorption (%ABS) was also calculated for the tested compounds (16).

$$\%ABS = 109 - 0.345 \times TPSA \quad (1)$$

From the presented in **Table 3** values for LogP, it is seen that the compounds possess hydrophobic properties, which facilitate their passing through the cell membranes and contribute to a good absorption. The theoretical values for %ABS may be considered as additional confirmation of this observation. It is obvious from the obtained results that all the new compounds are in agreement with the limitations of Lipinski's Rule of Five, which makes them suitable for future optimizations.

Prediction of the Possible Pharmacological Effects

The diversity of possible drug targets is so enormous, that finding a common denominator and expressing molecule druglikeness by a single "magic number" is challenging. On the other hand, some simple count criteria are also with a relatively limit-

ed applicability and are useful only to discard obvious non-drugs.

In an attempt to predict the possible pharmacological effects of the newly synthesized compounds we applied the virtual screening or *in silico* screening approach, which uses computational chemistry techniques to analyze large chemical databases in order to identify possible new drug candidates.

Thus, ready-to-use virtual screening models for four important drug classes, namely GPCR ligands, ion channel blockers, kinase inhibitors, and nuclear receptor ligands were used as method for prediction of the possible pharmacological effect of the target compounds. For obtaining more detailed information on the possible activity the evaluated compounds (**Group 3**) were enriched by two groups of previously synthesized structures identified as **Group 1** (13) and **Group 2** (14) respectively.

The obtained results are presented on **Figures 6, 7, and 8** for **Group 1, 2, and 3**, respectively.

It is obvious from the obtained from the virtual screening results that the newly synthesized com-

Figure 6. Possible pharmacological effects of compounds enlisted in Group 1

Figure 7. Possible pharmacological effects of compounds enlisted in Group 2

Figure 8. Possible pharmacological effects of compounds enlisted in Group 3

pounds forming **Group 3** express highest activity towards all evaluated models. On the other hand, amongst the group, compounds **IIIa** and **IIIb** are outlined with highest effects as GPCR ligands and enzyme inhibitors.

In general, all structures containing N-pyrrolyl hydrazone groups have been determined to have highly underlined GPCR ligand and enzyme inhibitory effects.

CONCLUSION

One new N-pyrrolyl hydrazide and four new hydrazones have been synthesized. The structures of the new compounds were elucidated by IR and ^1H NMR spectral data. The purity of the obtained compounds was proven by the corresponding melting points and TLC characteristics. Lipinski's Rule of Five parameters were applied for preliminary evaluation of the pharmacokinetic properties of the target molecules. Compounds **Ia-c** and **IIa-c** follow all Lipinski's parameters, which is a prerequisite for good pharmacokinetics. This conclusion is confirmed by the near to 100% values of % ABS. This makes the obtained compounds suitable for further optimizations. On the other hand, using the *in silico* screening approach for prediction of the possible pharmacological effect was determined, that all compounds express good activity towards GPCR ligands and as enzyme inhibitors, where compounds **IIIa** and **IIIb** are showing highest effects.

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REFERENCES

1. Bhardwaj V, Gumber D, Abbot V, Dhimana S, Sharma P. Pyrrole: a resourceful small molecule in key medicinal hetero-aromatics. *RSC Adv.* 2015; 5: 15233 .
2. Loudon MG. *Chemistry of Naphthalene and the Aromatic Heterocycles.* Organic Chemistry New York: Oxford University Press; 2002. p. 1135–6.
3. Armarego WLF, Chai CLL. *Purification of Laboratory Chemicals.* Elsevier. 2003. p. 608.
4. Jusélius J, Sundholm D. The aromatic pathways of porphins, chlorins and bacteriochlorins. *Phys. Chem. Chem. Phys.* 2000;2(10): 2145–51.
5. Cox M, Lehninger AL, Nelson DR. *Lehninger Principles of Biochemistry.* New York: Worth Publishers. 2000. ISBN 1-57259-153-6.
6. Popiolek L. Hydrazide-hydrazone as potential antimicrobial agents: overview of the literature since 2010. *MedChemRes.* 2017; 26(2): 287–301.
7. Lionta E, Spyrou G, Vassilatis DK, Cournia Z. Structure-based virtual screening for drug discovery: principles, applications and recent advances. *Curr Top Med Chem.* 2014; 14(16): 1923–38.
8. Ertl P, Rohde B, Selzer P. Fast calculation of molecular polar surface area as a sum of fragment-based contributions and its application to the prediction of drug transport properties. *J Med Chem.* 2000; 43: 3714–17.
9. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Delivery Rev.* 1997; 23:4–25.
10. Veber DF, Johnson SR, Cheng H-Y, Smith BR, Ward KW, Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. *J Med Chem.* 2002; 45: 2615–23.
11. Bijev A. Synthesis and preliminary screening of carbohydrazides and hydrazones of pyrrole as potential tuberculostatics. *Arzneim-Forsch/Drug Res.* 2006;56:96–103.
12. Lessigiarska I, Nankov A, Bocheva A, Pajeva I, Bijev A. 3D-QSAR and preliminary evaluation of anti-inflammatory activity of series of *N*-pyrrolyl carboxylic acids. *Il Farmaco.* 2005; 60(3): 209–18.
13. Vladimirova S, Bijev A. An access to new *N*-pyrrolyl carboxylic acids as potential COX-2 inhibitors via Paal-Knorr cyclization. *Heterocyclic Communications.* 2014; 20(2): 111–5.
14. Bijev A, Georgieva M. Pyrrole-based hydrazones synthesized and evaluated *in vitro* as potential tuberculostatics. *Letters in Drug Design&Discovery.* 2010;7:430–7.
15. Georgieva M, Bijev A, Prodanova P. Synthesis and comparative study of tuberculostatic activity of pyrrole-based hydrazones related to structural variations, *Pharmacia.* 2010; 57(1–4): 3–14.
16. Zhao YH, Abraham MH, Lee J, Hersey A, Luscombe CN, Beck G, et al. Rate-limited steps of human oral absorption and QSAR studies. *Pharm Res.* 2002; 19:1446.