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Original Article

Synthesis and Study of Antitumor Activity of Substituted Imidazolecarboxamides

Farzin Hadizadeh[a,b,*], Mohammad Ramezani[a,b], Zahra Tayarani[a], Maryam Eghbal[b]

[a]*Biotechnology and Pharmaceutical Research Center,*
[b]*Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran*

Abstract

In view of potential biological activities of small molecule polyamides, we synthesized some novel N-(2-aminoethyl)-1-benzyl-2- (alkylthio)-1H-imidazole-5-carboxamide (**7a,b**), and N-(2-(1-benzyl-2-(alkylthio) -1H-imidazole- 5-carboxamido) ethyl)-1-benzyl-2- (alkylthio)-1H-imidazole-5-carboxamides (**8a,b**) as antitumor agents. The antitumor activity of compounds **7a,b** and **8a,b** was studied at concentrations of 0.01, 0.1 and 1 mg/ml, using the potato disk bioassay technique. Vincristine at 0.25 mg/ml employed as positive control and had -67.24% tumor inhibition. Maximum % inhibition for potato tumors was found to be -75.52 for **7b** at 1 mg/ml.

Keywords: Antitumor; Imidazoecarboxamide; Potato disc.
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1. Introduction

In every human cell, genetic information is stored on a string-like DNA polymer which is approximately 1 meter in length and contains 3×10^9 units of information in the form of base pairs, within which is encoded approximately 80,000 to 100,000 genes or sets of instructions [1]. The specific interaction of proteins such as transcription factors with DNA controls the regulation of genes and hence cellular processes [2]. A wide variety of human conditions ranging from cancer to viral infection arise from malfunctions in the biochemical machinery that regulates gene-expression [3]. Designed small molecules which target specific DNA sequences offer a potentially general approach for gene-specific regulation [4]. Such molecules could be powerful therapeutics for combating life threatening diseases which result from misregulation in transcription.

The structures of two small molecules isolated from natural sources are shown in Figure 1. Among these DNA-binding molecules, distamycin is distinguished by its structural simplicity, having no chiral centers and an oligopyrrolecarboxamide core structure [5]. Structural studies of distamycin-DNA complexes reveal modular complexes in which adjacent pyrrolecarboxamides makes similar contacts with adjacent DNA base pairs. The relative simplicity of distamycin, with respect both to its chemical structure and its complexes

*Corresponding Author: Farzin Hadizadeh, Faculty of Pharmacy, Mashhad University of Medical Sciences, P.O.Box 91775-1365, Mashhad, Iran.
Fax: (98)511-8823251
E-mail: hadizadehf@mums.ac.ir

with DNA, guided the initial decision to use distamycin as a basis for designed polyamides having novel DNA-binding sequences specificity [6]. The structure of the complex between the minor groove binder netropsin and d(GGCC AATTGG) was recently determined via single-crystal X-ray techniques [7].

Polyamides containing N-methylpyrrole (Py) and N-methylimidazole (Im) amino acids provide a model for the design of artificial molecules for recognition of double helical DNA [8]. In this work, N-(2-aminoethyl)-1-benzyl-2-(alkylthio)-1H-imidazole-5-carboxamide (**7a,b**), and N-(2-(1-benzyl-2-(alkylthio)-^{1}H-imidazole-5-carboxamido) ethyl)-1-benzyl-2-(alkylthio)-1H-imidazole-5-carboxamide (**8a,b**) were synthesized as netropsin analogues. A test that has proven useful in monitoring the inhibition of crown-gall tumour is the potato disc assay [9-11]. It has been shown that inhibition of crown gall tumour initiation on potato discs has good agreement with compounds and plant extracts known to be active in the 3PS (*in vivo*, mouse leukaemia) antitumor assay [12]. In the present work, antitumor results are presented from such a screening of synthesized carboxamides (**7a,b, 8a,b**).

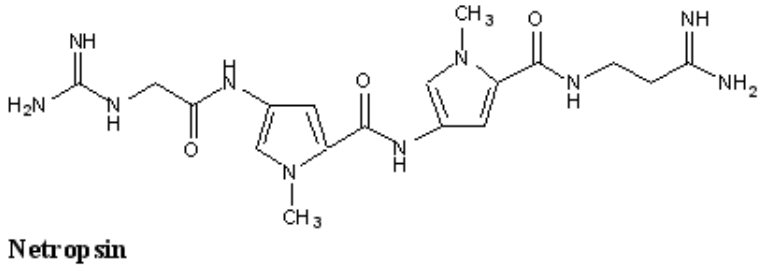
2. Experimental procedures

2.1. Chemistry

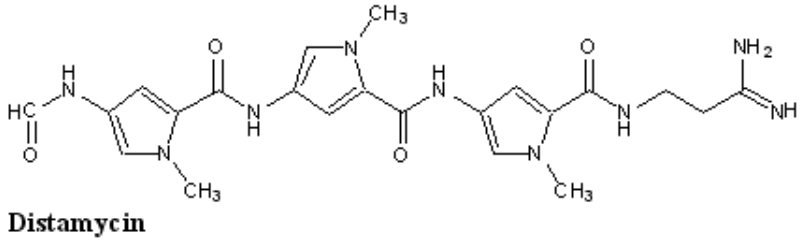
Melting points were determined on Capillary Electrothermal apparatus and are uncorrected. The IR spectra were measured on a Unicam SP-1100 spectrophotometer, using samples prepared as KBr disks. ^{1}H-NMR spectra were obtained on Bruker Ac-80 spectrophotometer and chemical shifts (d) are in ppm relative to internal tetramethylsilane. Elemental microanalyses were carried out with a Perkin-Elmer 240 °C apparatus and were within ±0.4% of the theoretical values for C, H, and N. All solvents and reagents were purchased from the Fluka, Aldrich or Merck Chemical Company. Compounds **1-6** were synthesized as it has been reported previously.

2.2. Synthesis of N-(2-aminoethyl)-1-benzyl-2-(methylthio)-1H-imidazole-5-carboxamide (7a)

To ethylenediamine (0.24 g, 4.0 mmol) in dry tetrahydrofuran (15 ml) was added dropwise at ice bath, crude acid halide **6a** (1.07 g, 4.0 mmol) in dry tetrahydrofuran (5 ml) and then stirred overnight at room temperature. The resulting mixture was basified by adding saturated solution of sodium bicarbonate and extracted with



Netropsin



Distamycin

Figure 1. Structure of two natural antitumor minor groove binding agents.

chloroform (3×100 ml). Chloroform was evaporated to give 0.87 g (3 mmol) of **7a** (75%); IR(KBr): ν 1680 cm^{-1}(C=O); ^{1}H-NMR ($CDCl_3$): δ7.80 (s, ^{1}H, H_4-imidazole), 7.30-7.27(m, 5H, arom), 5.50 (s, 2H, CH2N), 3.70(m, 4H, NCH_2CH_2N), 2.67ppm(s, 3H, CH3).

Anal. Calcd. for $C_{14}H_{18}N_4OS$: C, 57.91; H, 6.25; N, 19.29. Found: C, 57.71; H, 6.35; N, 19.09%.

*2.3. Synthesis of N-(2-aminoethyl)-1-benzyl-2-(ethylthio)-1H-imidazole-5-carboxamide hydrochloride (**7b**)*

It was prepared as described for **7a**, yield 65%; IR (KBr): ν 1680cm^{-1} (C=O); ^{1}H-NMR ($CDCl_3$): δ7.8 (s, 1H, H_4-imidazole), 7.5-7.0 (m, 6H, arom, NH), 5.47(s, 2H, CH_2N), 3.70 (m, 4H, NCH_2CH_2N), 3.14 (q, 2H, CH_2S), 1.28ppm (t, 3H, CH_3).

Anal. Calcd. for $C_{15}H_{20}N_4OS$: C, 59.18; H, 6.62; N, 18.41. Found: C, 59.39; H, 6.74; N, 18.30%.

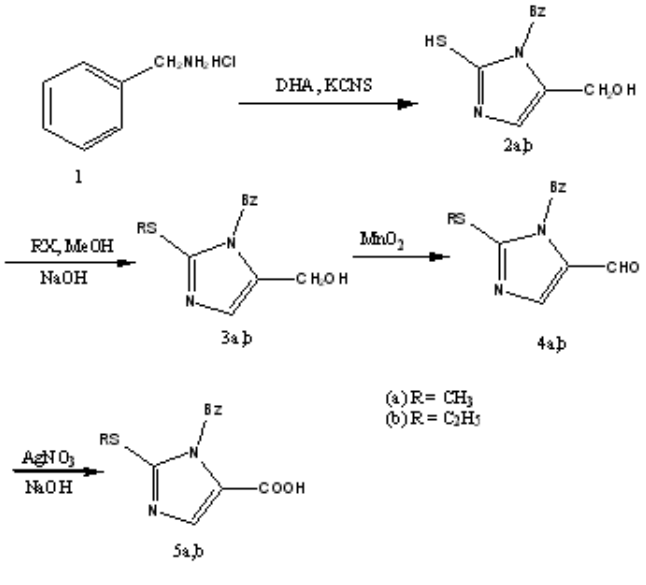
*2.4. Synthesis of N-(2-(1-benzyl-2-(methylthio)-1H-imidazole-5-carboxamido) ethyl)-1-benzyl-2-(methyl thio)-1H-imidazole-5-carboxamides (**8a**)*

To crude viscous liquid acid halide **6a** (1.07 g, 4.0 mmol) in dry tetrahydrofuran (15 ml) was added dropwise at ice bath, ethylenediamine (0.12 g, 2.0 mmol) in dry tetrahydrofuran (5 ml) and then stirred overnight at room temperature. The resulting mixture was basified by adding saturated solution of sodium bicarbonate and extracted with chloroform (3×100 ml). The combined organic layers were dried (Na_2SO_4) and evaporated to dryness. The crude was purified by silica gel column chromatography ($CHCl_3$/EtOH = 90:10) to afford 0.84 g (1.61 mmol) of **7a**, yield 40%; IR (KBr): ν 1713 cm^{-1} (C=O); ^{1}H-NMR (DMSO-d6): δ 7.80 (s, 2H, H4-imidazole), 7.30-7.27 (m, 12H, arom, NH), 5.5 (s, 4H, CH2N), 3.7 (s, 4H, NCH2CH2N), 2.7ppm (s, 3H, CH3).

Anal. Calcd. for $C_{26}H_{28}N_6O_2S_2$: C, 59.98; H, 5.42; N, 16.14. Found: C, 59.77; H, 5.53; N, 16.14%.

*2.5. Synthesis of N-(2-(1-benzyl-2-(ethylthio)-1H-imidazole-5-carboxamido)ethyl)-1-benzyl-2-(ethylthio)-1H-imidazole-5-carboxamides (**8b**)*

It was prepared as described from **6b** for **7a**, yield 35%; IR(KBr): ν 1700 cm^{-1}(C=O);



Scheme 1. Synthesis of compounds 2 to 5.

^{1}H-NMR (DMSO-d_6): δ 7.8-7.0 (m, 14H, arom, H_4-imidazole, NH), 5.47 (s, 4H, CH2N), 3.5-3.0 (m, 8H, NCH_2CH_2N, CH_2S), 1.2ppm (t, 3H, CH_3).

Anal. Calcd. for $C_{28}H_{32}N_6O_2S_2$: C, 61.29; H, 5.88; N, 15.32. found: C, 61.42; H, 5.65; N, 15.13%.

2.6. Screening with the potato disc assay

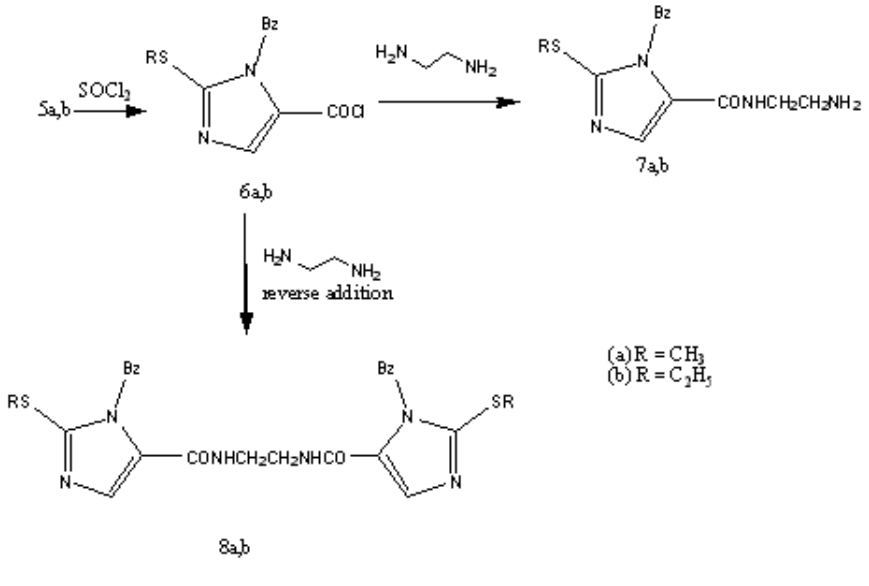
Fresh, disease-free potatoes were obtained from a local market. Tubers of moderate size were surface sterilized by immersion in sodium hypochlorite 0.1% for 20 min. Ends were removed and the potatoes were soaked for an additional 10 min. in sodium hypochlorite solution. A core of the tissue was extracted from each tuber with a surface-sterilized 1.0 cm cork borer. Pieces of 2 cm were removed from each end and discarded. The remainder of the cylinder was cut into 0.5 cm discs with a surface-sterilized scalpel. The discs were then transferred to agar plates (1.5 g of agar dissolved in 100 ml double distilled water (DDW), autoclaved for 20 min. at 121 °C, 20 ml poured into each Petri dish). Each plate contained 5 discs and 3-5 plates, were used for each sample dilution.

Agrobacterium tumefaciens (ATCC 23341) was cultivated in Soybean Casein Digest Agar. For inoculation of the potato discs, 48 h broth culture containing 5×10^9 cells/ml were used. Samples were dissolved in 5% DMSO, filter sterilized, diluted and mixed with the bacterial culture for inoculation.

The potato discs were incubated for 20 days at 25 °C incubator, after which Lugol's solution (I_2/KI) was added, the tumor counts were made and compared with negative controls (bacterial suspension containing 5% DMSO). The results were expressed as ± percentage versus the number of tumors on the control discs. Significant activity was indicated by consistent negative values of ca. 20% or greater inhibition. Vincristine was used as positive control.

%inhibition = [(average number of tumors in sample - average number of tumors in negative control) average number of tumors in negative control]×100

Minimum inhibitory concentration (MIC) of samples on *A. tumefaciens* was determined by microplate MTT-based method in nutrient broth. Gentamicin was used as positive control.



Scheme 2. Synthesis of compounds 7 and 8.

3. Results and discussion

3.1. Chemistry

Compounds **2-6** were synthesized as it has been previously reported [11]. Benzylamine hydrochloride (**1**) was stirred with 1,3-dihydroxyacetone dimmer and potassium thiocyanate to give 5-hydroxymethyl-2-mercapto-1-benzylimidazole (**2**). Subsequent alkylation of compound **2** with alkyl halides afforded 2-alkylsulfanyl-1-benzyl-5-hydroxy-methylimidazole (**3**). Oxidation of **3** with manganese dioxide gave **4**, which was further oxidized by boiling in alkaline solution of silver nitrate to give 2-alkylsulfanyl-1-benzylimidazole-5-carboxylic acid (**5**) (Scheme 1).

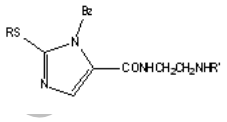
Compound **5** was converted to its acid halide (**6**), which was then added dropwise to ethylenediamine to give N-(2-aminoethyl)-1-benzyl-2-(alkylthio)-1H-imidazole-5-carboxamide (**7a,b**) (Scheme 2). Reverse addition of ethylenediamine to acid halide (**6**) afforded N-(2-(1-benzyl-2-(alkylthio)-1H-imidazole-5-carboxamido)ethyl)-1-benzyl-2- (alkylthio)-1H-imidazole-5-carboxamides (**8a,b**) (Scheme 2).

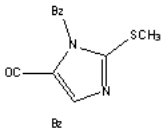
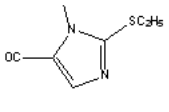
The proposed structure of compounds was confirmed by IR and ^{1}H NMR spectroscopy. Interpretation of the ^{1}H NMR spectra was base on the chemical shifts, multiplicities, and integral intensities of the signals.

3.2. Biological assay

Minimum inhibitory concentration (MIC) of samples on *A. tumefaciens* was found to be greater than 1 mg/ml, and at lower concentrations formazan pink color developed after MTT addition that did not differ with that of negative control, indicating no inhibition for *A. tumefaciens* growth. The concentrations above the MIC would produce false positive results in potato disc assay due to the antibacterial activity of the compounds. Therefore, dilutions below MIC including 0.01, 0.1 and 1 mg/ml were used for potato disc assay. The results are shown in Table 1. Vincristine at 0.25 mg/ml was employed as positive control and caused -67.24% inhibition. Compounds **7a** and **8a** had stimulatory effect on tumors at 1 mg/ml with positive values as +37.5 and +15.75, respectively. All compounds at other

Table 1: Antitumor activity of compounds 7a-d, 8a-d on potato disc model.



Compound	R	R'	Concentration (mg/ml)	Inhibition (%)
7a	CH_3	H	0.01	-55.1
			0.1	-49.66
			1	+37.5
7b	C_2H_5	H	0.01	-16.4
			0.1	-32.88
			1	-75.52
8a	CH_3		0.01	-18.4
			0.1	-12.18
			1	+15.75
8b	C_2H_5		0.01	-47.32
			0.1	-22.3
			1	-12.47
Vincristine	-	-	0.25	-67.24

concentrations had negative %inhibition, indicating tumor inhibition activity. Compound **7b** at 1mg/ml caused -75.52% tumor inhibition with highest activity among compounds tested.

Acknowledgements

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