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Full Paper

Simple and convenient synthesis of the bladder cancer biomarker: Nicotinuric acid

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Abstract

The present work describes a simple and convenient synthesis of nicotinoylglycine (nicotinuric acid) and some other aroylglycines such as substituted pyrazoloylglycines starting from their corresponding carboxylic acids. These acids were converted *via* two different methodologies, into their corresponding acid azides which were reacted with glycine in alkaline medium to give the target aroylglycines in very good yield. The use of the prepared nicotinuric acid as biomarker for early detection of bladder cancer will be the subject of a next publication.

Keywords: bladder cancer, biomarkers, aroylglycines, nicotinoylglycine, pyrazol-4-oylglycine.

1. Introduction

Bladder cancer (BC) is the second most common human malignancy affecting the urinary system and one of the deadliest cancers worldwide [1]. Its incidence continues to rise and mortality rates have remained unchanged over the past 3 decades [2]. BC ranks ninth in worldwide cancer incidences. It is the seventh most common cancer in males and the 17th most common cancer in females [3]. Males are 2.5–5 times more likely to develop this malignancy than females [4]. Incidence of bladder cancer increases with age and peaks at the sixth and seventh decades of life [5]. Globally, the incidence of bladder

cancer varies significantly, with Egypt and Western Europe while North America having the highest incidence rates and Asian countries having the lowest rates [6]. In the United States for 2012, it was estimated that 73,000 new cases would be diagnosed and 15,000 people would die from the disease [7]. In Egypt, according to the WHO data published in 2017, incidence rate of bladder cancer reached 4757 cases or 0.93% of the total deaths [8]. BC is the most expensive solid tumor to treat, because of its high recurrence rate and the need of continued surveillance, with an estimated annual cost of $3.7 billion in the United States [9]. Risk factors associated with the development of BC include carcinogens in tobacco smoke, and to a lesser extent exposure to chemical compounds in the chemical and rubber industries [10]. The success of BC treatment depends mainly on early detection [11]. Early detection of bladder cancer may improve patient prognosis and decrease the need for cystectomy by identifying tumors before they become muscle invasive [12]. Cystoscopy and voided urinary cytology are standards for the primary detection and follow-up of BC. Cystoscopy is invasive, while urinary cytology is less invasive but more specific and has limited sensitivity, especially for low-grade disease [13]. An effective biomarker for bladder cancer could lower costs, reduce the use of cystoscopy and identify earlier stage tumors. The ideal bladder cancer biomarker would be noninvasive, easy to use, reliable and efficacious. High sensitivity is necessary for surveillance as missed tumors put the patient at risk for disease progression and mortality and high specificity is needed as false positives would require invasive cystoscopic evaluation [12]. This has led to intense research to identify new diagnostic urinary biomarkers. Numerous urine-based biomarkers have shown an improved sensitivity compared to urinary cytology, but frequently with lower specificity [14-18]. Accordingly, it would be of interest to synthesize the highly expensive nicotinoylglycine (nicotinuric acid) as a biomarker for early detection of bladder cancer using a simple and efficient methodology. Also it would be beneficial to apply this methodology to synthesize other aroylglycines starting from their corresponding carboxylic acids.

2. Results and discussion

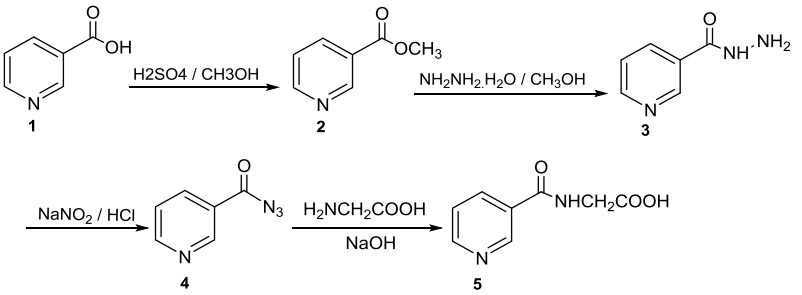
As it is known, cytology and cystoscopy are still standards for the detection of bladder cancer. However, the development of more reliable and less invasive techniques for early detection of such a cancer is always demanded [19]. It was fond that acylglycines are minor metabolites of fatty acids. The urinary excretion of certain acylglycines is increased in case of several inborn errors of metabolism. Accordingly, it deemed of interest, in our present work, to synthesize some aroylglycines such as nicotinoylglycine, and substituted pyrazoloylglycines for the sake of their use as potential urinary bladdercancer biomarkers.

We have attempted several trials for the synthesis of nicotinoylglycine **(5)** using nicotinic acid as a starting material. The acid was converted into its acid chloride, then the reaction of the acid chloride with glycine was attempted under different reaction conditions where no satisfactory result was obtained, However, the only successful methodology could be achieved by converting nicotinic acid into its methyl ester **2**, which was treated with hydrazine

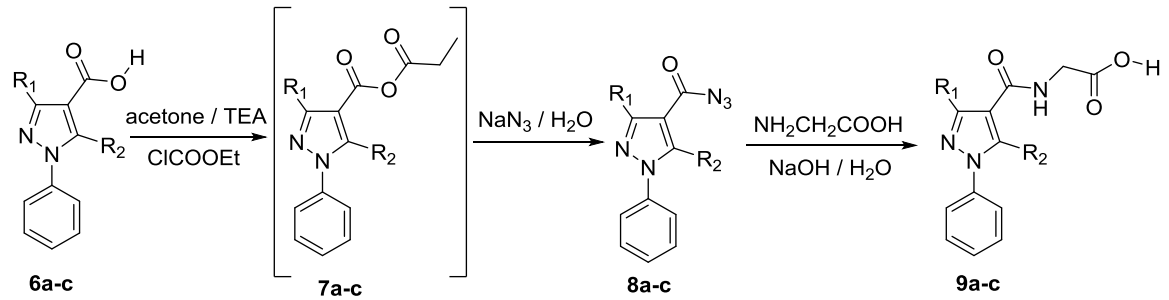
hydrate in boiling ethanol to give the acid hydrazide **3**. The latter compound afforded the acid azide **4** under diazotization reaction conditions. Finally, the interaction of **4** with glycine at room temperature in an alkaline solution gave the target nicotinoylglycine **(5)** in an excellent yield (**Scheme 1**)**.** It is worthy to mention that our methodology gave almost quantitative yield of the product, it proved to be simple, convenient and very low-cost methodology.

The same methodology was applied to the synthesis of the pyrazol-4-oylglycines **9a-c** starting with the pyrazole-4-carboxylic acid **6a-c** [20]. However, the acid azide **8a-c** could be obtained in a one-pot synthesis *via* the conversion of the acid **6a-c** into the corresponding mixed anhydride **7a-c** followed by treatment with sodium azide giving acid azide **8a-c**. This acid azide **8a-c** was finally interacted with glycine in an alkaline solution at rt. giving the pyrazol-4-oylglycines **9a-c** (**Scheme 2**).The IR spectra of these compounds showed the disappearance of the absorption bands of the azide groups, and the appearance of bands within 3435-3270 cm^{-1} (ν NH), in addition to bands within 3136 – 2508 cm^{1} (ν OH of the carboxylic acid function) and bands within 1741-1691 cm^{-1} (νC=O), meanwhile their ^{1}H-NMR spectra showed characteristic signals assignable to CH_2, NH, and OH protons (cf. experimental part).

Preliminary evaluation of the synthesized nicotinuric acid as biomarker for the early detection of bladder cancer was highly promising. It is now under extensive study to evaluate their use in detecting bladder cancer in the urine of a number of patients from South Egypt Cancer Institute, Assiut Universiy- Egypt. The results will be published in a future publication.



Scheme 1. Synthetic pathway of nicotinoylglycine (**5**)**.**



a; R_1 = Ph, R_2 = H
b; R_1 = Ph, R_2 = Cl
c; R_1 = CH_3, R_2 = Cl

Scheme 2. Synthesis of 3,5-disubstituted 1-phenyl-1*H*-pyrazol-4-oylglycine derivatives **9a-c.**

3. Experimental

All melting points are uncorrected and measured on Stuart melting point apparatus (Bibby Scientific) SMP3. The elemental analysis was carried out at the Micro Analytical Center of Chemistry Department, Assiut University. The FT IR spectra were recorded using a Shimadzu 470 IR spectrophotometer using the KBr wafer technique. ^{1}H NMR spectra were obtained on a Bruker 400 MHz spectrometer in DMSO-d_6 using Me_4Si as internal standard and chemical shifts were expressed as ppm. All reagents being of A.R. grade, were purchased from Merck, Sigma Aldrich and Loba chemical companies.

3.1. Methyl nicotinate (2)

A mixture of nicotinic acid (30 g, 0.2 mol), methanol (90 ml) and conc. H_2SO_4 (17 ml) was heated under refluxed for 12 h. The reaction mixture was then cooled and neutralized with a solution of sodium carbonate, followed by extraction with dichloromethane. The aqueous layer was extracted once more, and the combined organic layer was washed with brine and dried over sodium sulfate. The solvent was distilled, filtered and white crystals obtained. Yield: 21.3 g (64 %); mp, 38-39°C [38°C, 21]. IR (**ν**, cm^{-1}): 3057 (CH aromatic), 2956 (CH aliphatic), 1727 (C=O), 1590 (C=N).

3.2. Nicotinic acid hydrazide (3)

A mixture of methyl nicotinate (10 g, 0.07 mol) and hydrazine hydrate (8 ml, 80%) in ethanol (50 ml) was heated under reflux for 3 h. After cooling, the reaction mixture was concentrated under reduced pressure and the solid obtained was recrystallized from ethanol. White crystals, yield: 8.01 g (80%); mp, 159-160 °C [21]. IR (**ν**, cm^{-1}): 3322-3207 (NH, NH_2), 3018 (CH aromatic), 1673 (C=O), 1596 (C=N).

3.3. Nicotinoyl azide (4)

A cold solution of $NaNO_2$ (3.5 g, 0.05 mol) in water (20 mL) was added dropwise with stirring within 30 min to a cold solution of nicotinic acid hydrazide (3.5 g, 0.03 mol) in diluted HCl (4 mL conc. HCl /10 mL H_2O) at 5-10°C. Stirring was continued for 1.5 h under that temperature. The pH of the reaction mixture was then adjusted to pH 7 by dropwise addition of Na_2CO_3 solution (20%) followed by extraction with ether. The combined organic layer was washed brine and dried over Na_2SO_4. Evaporation of the solvent gave a solid product which was recrystallized from water to give white crystals of nicotinoyl azide, yield: 2.73 g (72%); mp, 49-50 °C [48-50°C, 22]. IR (**ν**, cm^{-1}): 3025 (CH aromatic), 2179 (N_3), 1690 (C=O), 1585 (C=N).

3.4. Nicotinoylglycine (5)

Nicotinoyl azide (2.5 g, 0.02 mol) was added in small portions to a solution of glycine (1.9 g, 0.03 mol) in NaOH (17 ml, 1N) with vigorous stirring. Additional NaOH solution was added to the reaction mixture at intervals to maintain the pH 8-9 and stirring was continued for 12 h at rt. The reaction mixture was then evaporated till dryness under reduced pressure. The residue obtained was acidified using 2N HCl solution to pH 4 in an ice bath. The solid precipitate of nicotinoylglycine was filtered and recrystallized from water to give white crystals. Yield: 2.81 g (92 %); mp: 254-255 °C. IR (**ν**, cm^{-1}): 3312 (NH), 3034 (CH aromatic), 2938 (CH aliphatic), 2736-2233 (OH of COOH), 1724 (C=O acid), 1632 (C=O amide), 1600 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6),**δ** (ppm): 3.98 (s, 2H, CH_2), 7.51-7.54 (dd, 1H, pyridine-

H5), 8.21 (s, 1H, NH), 8.23 (dd, 1H, pyridine-H4), 8.72 - 8.74 (dd, 1H, pyridine-H6), 9.00 - 9.04 (s, 1H, pyridine-H2), 12.61 (s, 1H, OH). Anal. Calcd. for $C_8H_8N_2O_3$ (180.16): C, 53.33; H, 4.48; N,15.55%. Found: C, 53.39; H, 4.56; N,15.46%.

3.5. Synthesis of 3,5-disubstituted 1-phenyl-1*H*-pyrazole-4-carboxylic acid azide 8a-c:

3.5.1. 1,3-Diphenyl-1H-pyrazole-4-carboxylic acid azide [20] (8a):

1,3-Diphenyl-1*H*-pyrazole-4-carboxylic acid **(6a**) (1 g, 3.7 mmol) was dissolved in 100 ml of acetone at 0 °C. Triethyl amine (0.8 ml, 5.7 mmol) was added dropwise at 0 °C, after 30 min at 0 °C ethyl chloroformate (0.5 ml, 5.2 mmol) was added at 0 °C dropwise. After 30 min at 0 °C add sodium azide (0.6 g, 9.2 mmol) in 10 ml water, stirring at 0 °C for 2 hours and pour on cold water and filtered. Yeild: 0.9 g (82%); mp: 180-182 °C. IR (**ν**, cm^{-1}): 3056 (CH aromatic), 2162 (N3), 1664 (C=O), 1600 (C=N).

3.5.2. 5-Chloro-1,3-diphenyl-1H-pyrazol-4-oylazide (8b):

This was obtained from 5-Chloro-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid **(6b)** (1 g, 3.3 mmol) as described for **8a**. Yield: 0.18g (61%); mp: 298-301 °C. IR (**ν**, cm^{-1}): 3060 (CH aromatic), 2280 (N3), 1648 (C=O), 1595 (C=N).

3.5.3. 5-Chloro-3-methyl-1-phenyl-1H-pyrazole-4-carboxylic acid azide (8c):

This was obtained from 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**6c)** (1 g, 4.2 mmol) as described for **8a**. Yield: 0.5g (45%); m.p: 259-261 °C. IR (**ν**, cm^{-1}): 3036 (CH aromatic), 2925 (CH aliphatic), 2162 (N3), 1681 (C=O), 1594 (C=N).

3.6. Synthesis of 3,5-disubstituted 1-phenyl-1*H*-pyrazol-4-oylglycine derivatives 9a-c:

3.6.1. 1,3-Diphenyl-1H-pyrazol-4-oylglycine (9a):

1,3-Diphenyl-1*H*-pyrazole-4-carboxylic acid azide **(8a)** (0.25 g, 0.86 mmol) was added in small portions to a solution of glycine (0.18 g, 2.3 mmol) in NaOH solution (1N, 4 ml), and additional 1N NaOH was added to the solution at intervals to maintain the pH 8-9. with vigorous stirring for 24 h. The reaction solution was evaporated till dryness under reduced pressure. The residue obtained was acidified by addition of 2N HCI till pH 4 in an ice bath. The precipitated solid was filtered, washed with cooling water and recrystallized from water. Yield: 0.17g (61%); mp: 200-203 °C. IR (**ν**, cm^{-1}): 3435 (NH), 3136-2508 (OH), 3052 (CH aromatic), 2922 (CH aliphatic), 1691 (C=O acid), 1670 (C=O amide), 1598 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) **δ** (ppm): 3.93 (s, 2H, CH_2), 7.44-7.89 (m, 10H, Ar-H), 7.98 (s, 1H, pyrazole-H5), 8.48 (s, 1H, NH), 9.04 (s, 1H, OH). Anal. Calcd. for $C_{18}H_{15}N_3O_3$ (321.34): C, 67.28; H, 4.71; N, 13.08%. Found: C, 67.24; H, 4.69; N, 13.12%.

3.6.2. 5-Chloro-1,3-diphenyl-1H-pyrazol-4-oylglycine (9b):

This was obtained from 5-Chloro-1,3-diphenyl-1*H*-pyrazole-4-carboxylic acid azide **(8b)** (0.25 g, 0.7 mmol) as described for **9a**. Yield: 0.07g (64%); mp: 215-217 °C. IR (**ν**, cm^{-1}): 3270 (NH), 3109-2730 (OH), 3062 (CH aromatic), 2920 (CH aliphatic), 1736 (C=O acid), 1638 (C=O

amide), 1594 (C=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.93 (s, 2H, CH_2), 7.39-7.99 (m, 10H, Ar-H), 8.64 (s, 1H, NH), 9.05 (s, 1H, OH). Anal. Calcd. for $C_{18}H_{14}ClN_3O_3$ (355.78): C, 60.77; H, 3.97; Cl, 9.96; N, 11.81%. Found: C, 60.75; H, 4.01; Cl, 9.93; N, 11.85%.

3.6.3. 5-Chloro-3-methyl-1-phenyl-1H-pyrazol-4-oylglycine (9c):

This was obtained from 5-Chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid azide **(8c)** (0.25 g, 0.9 mmol) as described for **9a**. Yield: 0.15g (53%); mp: 196–199 °C. IR (**ν**, cm^{-1}): 3418 (NH), 3084-2515 (OH), 3050 (CH aromatic), 2931 (CH aliphatic), 1741 (C=O acid), 1679 (C=O amide), 1597 (C=N). ^{1}H-NMR (400 MHz, DMSO-d_6) δ (ppm): 2.51 (s, 3H, CH_3), 3.76 (s, 2H, CH_2), 7.40-7.86 (m, 5H, Ar-H), 7.99 (s, 1H, NH), 8.29 (s, 1H, OH). Anal. Calcd. for $C_{13}H_{12}ClN_3O_3$ (293.71): C, 53.16; H, 4.12; Cl, 12.07; N, 14.31%. Found: C, 53.19; H, 4.15; Cl, 12.03; N, 14.28%.

References:

1. M. Burger, J. W. Catto, G. Dalbagni, H. B. Grossman, H. Herr, P. Karakiewicz and Y. Lotan,Eur. Urol. 63 (2013) 234.
2. Z. Huang, L. Lin, Y. Gao, Y. Chen, X. Yan, J. Xing and W. Hang, Mol. Cell Proteomics, mcp-M111 (2011).
3. C. Murta-Nascimento, B. J. Schmitz-Drager, M. P. Zeegers, G. Steineck, M. Kogevinas, F. X. Real and N. Malats, World J. Urol. 25 (2007) 285.
4. M. C. Yu and R. K. Ross, In: Petrovich Z, Baert L, Bardy L, editors Carcinoma of bladder Berlin, Springer, 1998, pp. 1–13 DOI: 10.1007/978-3-642-60258-0_1
5. W. U. Shipley, D. S. Kaufman and W. S. Dougal, In: DeVita VT Jr, Hellman S, Rosenberg SA, (ed) Cancer Principles and Practice Oncology, 7th edition. Philadelphia: Lippincott Williams and Wilkins pp. 1168–92 (2005).
6. J. Ferlay, F. Bray, P. Pisani and D. M. Parkin, IARC Cancer Base No. 5, Version 2.0. (2004).
7. R. Siegel, D. Naishadham and A. Jemal, CA Cancer J. Clin. 62 (2012) 10.
8. J. Ferlay, I. Soerjomataram, M. Ervik, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. M. Parkin, D. Forman and F. Bray, Int. J. Cancer136 (2015) 359.
9. K. D.Sievert, B. Amend, U. Nagele, D. Schilling, J. Bedke, M. Horstmann and A. Stenzl, World J. Urol. 27 (2009) 295.
10. S. Goodison, C. J. Rosser and V. Urquidi, Mol. Diagn. Ther.17 (2013) 71.
11. A. P. Mitra and R. J. Cote, Nat. Rev. Urol.7 (2010) 11.
12. A. Alvarez and V. B. Lokeshwar, Curr. Opin. Urol. 17 (2007) 341.
13. I. Karaoglu, A. G. van der Heijden and J. A. Witjes, World J. Urol. 32 (2014) 651.
14. A. M. Kamat, P. K. Hegarty, J. R. Gee, P. E. Clark, R. S. Svatek, N. Hegarty and L. C. Jenkins, Eur. Urol.63 (2013)4.
15. M. Horstmann, H. Bontrup, J. Hennenlotter, D. Taeger, A. Weber,B. Pesch and T. Bruning, World J. Urol. 28 (2010) 399.
16. M. Horstmann, T. Todenhofer, J. Hennenlotter, S. Aufderklamm, J. Mischinger, U. Kuehs and C. Schwentner, World J. Urol. 31 (2013) 935.

17. M. Miyake, S. Goodison, W. Rizwani, S. Ross, H. B. Grossman and C. J. Rosser, World J. Urol. 30 (2012) 869.
18. L. B. Tan, K. T. Chen, Y. C. Yuan, P. C. Liao and H. R. Guo, World J. Urol. 28 (2010) 117.
19. H. G. Van der Poel and F. M. Debruyne, Curr. Opin. Urol. 11 (2001) 503.
20. A. R. Farghaly, S. Esmail, A. Abdel-Hafez, P. Vanelle and H. El-Kashef,. Arkivoc, 7(2012)228.
21. M. B. Deshmukh, S. H. Patil and C. S. Shripanavar, J. Chem. Pharm. Res.4 (2012) 326.
22. Z. Z. Qin, F. B. Zhang, T. N. Jiang, M. J. Li, R. X. Wang And Y. L. Li, Chem. Res. Chin. Univ. 24 (2008) 496.