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Research paper

Molecular modeling and snake venom phospholipase A_2 inhibition by phenolic compounds: Structure–activity relationship

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ABSTRACT

In our earlier study, we have reported that a phenolic compound 2-hydroxy-4-methoxybenzaldehyde from *Janakia arayalpatra* root extract was active against Viper and Cobra envenomations. Based on the structure of this natural product, libraries of synthetic structurally variant phenolic compounds were studied through molecular docking on the venom protein. To validate the activity of eight selected compounds, we have tested them in *in vivo* and *in vitro* models. The compound **21** (2-hydroxy-3-methoxy benzaldehyde), **22** (2-hydroxy-4-methoxybenzaldehyde) and **35** (2-hydroxy-3-methoxybenzylalcohol) were found to be active against venom-induced pathophysiological changes. The compounds **20**, **15** and **35** displayed maximum anti-hemorrhagic, anti-lethal and PLA_2 inhibitory activity respectively. In terms of SAR, the presence of a formyl group in conjunction with a phenolic group was seen as a significant contributor towards increasing the antivenom activity. The above observations confirmed the anti-venom activity of the phenolic compounds which needs to be further investigated for the development of new anti-snake venom leads.

1. Introduction

Snake envenomation is one of the neglected problems of the world. Antiserum still remains the only therapeutic agent available for snakebite treatment. In the remote and rural areas of many developing countries, antiserum is generally not easily available. The antiserum sometimes does not produce enough protection against venom induced hemorrhage, necrosis, nephrotoxicity and hypersensitive reactions [1–3]. Further development of antiserum in animal is time consuming, costly and requires strict cold chain conditions. To overcome these factors search for natural/synthetic molecules as an alternate source of antidote is an urgent requirement. The development of stable and effective venom neutralizing drug/antidote is being ventured throughout the World. One of the strategies could be to develop a molecule from the natural/synthetic sources to combat the effect of venom partially or completely which may help in improving the chances of survival of snake bite victims. Venom neutralizing drugs may prove to be more effective life saver [4]. Development of such molecules is vital to address the cases when identification of the snake is difficult, specific treatment is unavailable and the health centre is far away.

Many plants and plant products have been used in folk medicines as antidote against snakebite [5–8]. Preliminary investigations have suggested that several classes of constituents such as steroids, terpenoids, alkaloids and glycosides from plant origin are capable of neutralizing snake venom induced pathophysiological changes [9–16]. More than 800 plant species have been screened for the anti-snake venom activity [15], however, very few plant extracts have shown significant protection against snake-venom and none of the pure isolates has displayed equivalent activity [4,17,14,18,19]. Several workers have also reported

Abbreviations: ASP, Aspartate; $CDCl_3$, Deuterated chloroform; DMSO, Dimethyl sulfoxide; FT-IR, Fourier transform-Infrared spectroscopy; GLY, Glycine; LD_{50}, Lethal Dose 50 (dose required to kill 50 percent of a population of test animals); MCDP/S, Minimum clotting dose of plasma/serum; MDD, Minimum defibrinogenating dose; MHD, Minimum hemorrhagic dose; MHz, Megahertz; NMR, Nuclear magnetic resonance; PLA_2, Phospholipase A_2; ppm, part per million; SAR, Structure–activity relationship; TLC, Thin layer chromatography; TMS, Tetramethylsilane.

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natural as well as synthetic compounds as active inhibitors of snake venom phospholipase A_2 (PLA_2) [20–22]. Phenolic compounds such as phenolic acids (hydroxy benzoic acid or benzaldehydes, cinnamic acid), flavonoids, isoflavonoids, coumarins, pterocarpans, phenolic glycosides and tocopherols have been reported to display protection against variety of snake venoms [9,17,23]. In our earlier study, a phenolic compound 2-hydroxy-4-methoxy-benzoic acid was shown to possess anti-venom and antioxidant activity [4,17]. Through *in silico* approach we identified a natural compound 2-hydroxy-4-methoxy-benzaldehyde (**21**) as snake venom PLA_2 inhibitors [IOXL] [24]. The natural product (**21**) was isolated from the root extracts of *Janakia arayalpathra* which is used in folk medicine in Western India as an antidote of snake bite. It was also reported in the literature as an immunomodulator [5,6] and potent inhibitor of tyrosinase, which is essentially indicative of its binding capabilities to specific receptor proteins [25]. In the present study, we examined several analogs of phenolic compounds against Viper and Cobra venom induced pathophysiological changes which were found to be effective both in wet lab and in *in silico* models.

2. Materials and methods

2.1. Materials

Unless otherwise stated, all reagents for chemical synthesis were purchased from Sigma–Aldrich and used without further purification. The solvents used in reactions were distilled and dried before use. All the material either natural or synthetic was in pure form. The purity of the natural as well as synthetic compounds was found to be more than 95%. All reactions were monitored by TLC on 0.25 mm silica gel 60 F254 plates coated on Aluminum sheet (E. Merck). ^{1}H NMR and ^{13}C NMR spectra were recorded on Brucker Avance DPX-200 instrument at 200 MHz and 500 MHz respectively using $CDCl_3$ as solvent with TMS as internal standard. Chemical shift is expressed in δ (ppm) and coupling constant in Hertz.

2.2. Chemistry

The melting point of the compounds isuncorrected. Reagents, chemicals and solvents were purchased from Sigma Aldrich and E Merck. TLCs were run on 0.25 mm silica gel 60 F_{254} plates (E. Merck) using UV light or Ceric ammonium sulfate solution for detection/visualization of the spots. The purification of the products was carried out by column chromatography over silica gel (60–120 mesh). NMR spectra were run on Bruker Avance DPX-200 at 200 MHz, IR on Bruker 270-30 FT-IR and Mass spectra on JEOL MSD 300. Elemental analysis was performed on ElementarVario EL-III. Anisaldehyde, 2-hydroxy-3-methoxybenzaldehyde, 3-methoxy-4-hydroxy-benzaldehyde, 3-hydroxy-4-methoxy-benzaldehyde, 1,2-dihydroxybenzene, 3,4-dihydroxybenzaldehyde, 2-hydroxybenzaldehyde, 3-ethoxy-4-hydroxy-benzaldehyde were purchased from Sigma–Aldrich.

2.2.1. Isolation and characterization of natural and synthetic compounds

The plant material of *Janakia arayalpatra* (root) was obtained from local market and was identified in the Department of Taxonomy, IIIM, Jammu.

2.2.1.1. Isolation of 2-hydroxy–4-methoxybenzaldehyde. Crushed dry roots of *J. arayalpatra* (500 g) was steam distilled for 6 h to get a pale yellowish liquid (1.4 ml, 0.28%) which on cooling afforded a solid mp 40–41 °C (Lit mp 40–42 °C). IR (KBr pellet): 3260, 3030, 2944, 1644, 1630, 1576, 1504, 144, 1366, 1336, 1296, 1222, 1204, 1164, 1136, 1020 and 798 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.82 (3H, s, Ar-OMe), 6.30 (1H, d, J = 2.2 Hz, Ar–H) 6.34 (1H, dd, J = 8 Hz & 2.2 Hz. Ar–H) 7.26(1H, d, J = 8 Hz, Ar–H), 9.72 (1H, s, –CHO), 11.33 (1H, s, OH). MS M$^+$ *m/z* (%) 152(11), 137(33), 123(27), 121(34), 82(10), 53(24).

2.2.1.2. Preparation of 2-hydroxy–4-ethoxybenzaldehyde. Anhydrous potassium carbonate (2 g) and ethyl iodide (0.5 ml) were added to 2,4-dihydroxy benzaldehyde (0.7 g, 5 mmol) acetone (30 ml) and the contents stirred for 12 h at room temperature. After the completion of the reaction as indicated by TLC, the mixture concentrated in vacuo and re-dissolved in benzene (3 × 10 ml). The organic layer concentrated and the residue chromatography on silica gel (60–120 mesh) using n-hexane: ethyl acetate mixture (46:4) as eluent to give JA-2 a crystallized solid (0.75 g, 45%) melting point 38–39 °C analyzed $C_9H_{10}O_3$. IR (KBr pellet): 3340, 3284, 1674, 1604, 1566, 1444, 1408, 1328, 1252, 1230, 1216, 1180, 1086, 1026, 916 and 846 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ1.45(3H,t, J = 6.8 Hz, CH_3–CH_2O), 4.17 (2H,q, J = 6.8 Hz, CH_3–CH_2O), 6.71 (1H, s, Ar–H),7.03 (1H, d, J = 8.5 Hz, Ar–H),7.41(1H, dd, J = 8.5 Hz & 2.2 Hz, Ar–H),9.8 (1H, s, Ar-CHO). MS M$^+$ *m/z* (%) at 166(100), 149(5), 137(9), 109(14), 108(13), 80(40), 79(31), 63(44).

2.2.1.3. Preparation of 2-hydroxy–4-propyloxybenzaldehyde. To 2, 4-dihydroxy benzaldehyde (0.7 g, 5 mmol) in acetone (30 ml) was added anhydrous potassium carbonate (2 g) and n-propyl bromide (0.5 ml) and the contents stirred for 12 h at room temperature. The contents concentrated in vacuo and re-dissolved in benzene (3 × 10 ml). The organic layer concentrated and the residue chromatography on silica gel (60–120 mesh) using n-hexane: ethyl acetate mixture (46:4) as eluent to give an oily compound (0.46 g, 52%) analyzed for $C_{10}H_{12}O_3$. IR (KBr pellet): 3384, 2932, 1668, 1634, 1578, 1498, 1454, 1428, 1374, 1336, 1292, 1260, 1216, 1170, 1114, 998, 826 cm^{-1}. ^{1}H NMR (200 MHz,$CDCl_3$): δ 1.0 (3H, t, J = 7.4 Hz, CH_3–CH_2),1.77–1.88 (2H, m, OCH_2–CH_2–CH_3), 3.97 (2H, t, J = 6.53 Hz,OCH_2–CH_2), 6.41 (1H, d, J = 2.02 Hz, Ar–H),6.5 (1H, dd, J = 8.6 & 2.0 Hz, Ar–H) 7.41 (1H, d, J = 8.6 Hz, Ar–H), 9.75 (1H, s, CHO). MS M$^+$ *m/z* (%) 180(100), 138(98), 120(16), 110(38), 109(15), 92(18), 82(12), 81(50), 69(35), 65(50).

2.2.1.4. Preparation of 2-hydroxy–4-butyloxybenzaldehyde. To 2, 4-dihydroxy benzaldehyde (0.7 g, 5 mmol) in acetone (30 ml) was added anhydrous potassium carbonate (2 g) and n-butyl bromide (0.5 ml) and the contents stirred for 12 h at room temperature. The contents concentrated in vacuo and re-dissolved in benzene (3 × 10 ml). The organic layer concentrated and the residue chromatographed on silica gel (60–120 mesh) using n-hexane: ethyl acetate mixture (46:4) as eluent to give an oily compound (0.53 g, 55%) analyzed for $C_{11}H_{14}O_3$. IR (KBr pellet): 3344, 2957, 1681, 1644, 1573, 1478, 1216, 1178, 1080, 914, 832 cm^{-1}. ^{1}H NMR (200 MHz,CDCl3): δ 0.98 (3H, t, J = 7.28 Hz, CH_3–CH_2) 1.43–150 (2H, m, CH_2–CH_2), 1.71–1,85 (2H, m, OCH_2–CH_2–CH_2), 4.0 (2H, t, J = 6.46 Hz, OCH_2–CH_2), 6.41 (1H, d, J = 2.1 Hz, Ar–H), 6.65 (1H, dd, J = 2.1 & 8.6 Hz, Ar–H), 7.42 (1H, d, J = 8.6 Hz, Ar–H), 9.7 (1H, s, CHO). MS M$^+$ *m/z* (%), 194(52), 151(2.6), 139(16), 138(100), 137(98), 110(13), 92(5), 81(16), 69(11), 65(19).

2.2.1.5. Preparation of 2-hydroxy–4-heptyloxybenzaldehyde. To 2, 4-dihydroxy benzaldehyde (0.7 g, 5 mmol) in acetone (30 ml) was added anhydrous potassium carbonate (2 g) and n-heptyl bromide (0.5 ml) and the contents stirred for 12 h at room temperature. The contents concentrated in vacuo and redissolved in benzene (3 × 10 ml). The organic layer concentrated and the residue chromatography on silica gel (60–120 mesh) using n-hexane: ethyl acetate mixture (46:4) as eluent to give an oily compound (0.58 g, 50%) analyzed for $C_{14}H_{20}O_3$. IR (KBr pellet): 3330, 3240, 2955, 1677,