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Full Length Article

Antimicrobial, analgesic, antioxidant and *in silico* study of synthesized salicylic acid congeners and their structural interpretation

Jyotirmaya Sahoo *, *Sudhir Kumar Paidesetty*

Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Siksha'O'Anusandhan University, Bhubaneswar Pin-751003, Odisha, India

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ABSTRACT

A series of azosalicylic acid analogs were newly synthesized by coupling various aryl and heteroarylamine functionalities with salicylic acid nucleus. All the synthesized compounds were structurally confirmed by various modern analytical methods. The said synthesized compounds were screened to investigate their antimicrobial, analgesic and antioxidant activities. The compounds **4e** and **4h** showed excellent significant antibacterial activity against most of the bacterial strains as no compounds showed significant antifungal activity against *Cryptococcus neoformans*. The bromine substituted molecule **4e** (4-bromo-3-methyl phenyl azosalicylic acid analog) showed the highest significant analgesic activity with 46.10% of inhibition. The results of *in vitro* antibacterial and analgesic activity were justified with the outcome of *in-silico* investigation. The results of biological activities were statistically interpreted. The compounds substituted with antipyrinylazo and 4-carboxy phenylazo moiety exhibited potential antioxidant activity.

1. Introduction

The writings of Greek physician Hippocrates revealed that the leaves and barks of willow tree were used as analgesic and antipyretic in early days. The active constituent responsible in this natural source, later identified as salicin, contains both sugar and aromatic component, initially called as spirasure and later salicylic acid. The *de-novo* synthesis of salicylic acid was first performed in 1852 and its structure was deduced as 2-hydroxy benzoic acid [1]. The salicylic acid derivatives exhibited antioxidant, antiproliferative [2] and cytotoxic activities [3]. The azo salicylic acid derivative sulfasalazine is a proven drug for the last 40 years which is effective against ulcerative colitis (inflammatory bowel disease) [4]. There has been an increase in the side-effects due to the sulfapyridine portion which acts as a carrier. The azo bond breaks due to the bacterial enzyme azo-reductase present at the site of lumen of the colon leaving the 5-aminosalicylic acid. The azobis-salicylic acid derivative olsalazine could be a better alternative for sulfasalazine.

* *Corresponding author.* Tel.: +91 09861433157.
E-mail address: jjyotisahoo@rediffmail.com (J. Sahoo).

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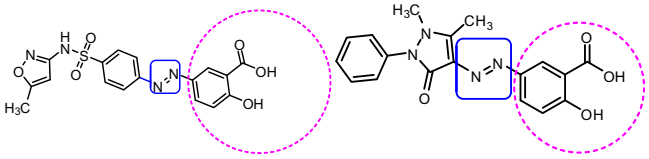
Literature survey supports that azo-salicylic acids have biological activity and also are useful precursors for the synthesis of anticarcinogenic, antiviral, antimicrobial and antimalarial agents [3]. Salicylates have analgesic effects similar to that of other NSAIDs to inhibit the enzyme cyclooxygenase (COX) [5]. NSAIDs inhibit both the activity of COX-1 and COX-2 and thereby synthesis of prostaglandin and thromboxane [6]. Literature support also suggests that bromine substituted molecules can show potential analgesic activity [7]. Further, literature survey indicates that pyrazolone nucleus is the key pharmacophore and is responsible for various pharmacological activities such as analgesic [8] and antimicrobial activity [9]. The N-phenyl substituted anthranyl congeners also have analgesic, antirheumatic and antiinflammatory activities [10]. The above information encouraged us to synthesize a new range of azo-salicylic acid congeners with different aryl and heteroaryl functionalities and to investigate the antibacterial, analgesic and antioxidant activities. The structures were confirmed by spectral characterization. The synthesized azosalicylic acid congeners act as ligands individually against the targeted proteins (PDB ID: 3SPU of NDM-1 and 1CX2 of COX-2) by computational docking method for the evaluation of antibacterial and analgesic activities respectively.

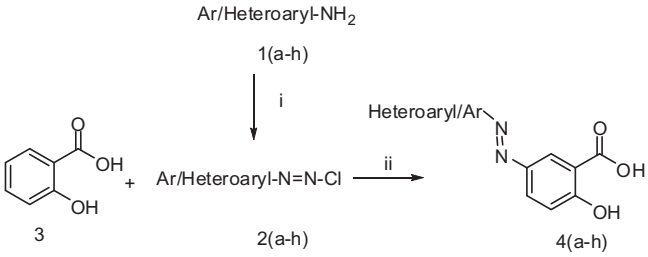
Structures of some newly synthesized azosalicylic acid congeners



2. Materials and methods

All the chemicals used in the present study were of synthetic grade and sourced from Merck Specialties Ltd. (Mumbai, India). The structural conformation of the synthesized compounds from salicylic acid is conducted by various modern analytical techniques viz. FT/IR (JASCO FT/IR 4100 Spectrophotometer using KBr disc), ^{1}H NMR (Bruker ^{1}H NMR 400 *MHz*) using TMS as an internal standard, LC–MS (Shimadzu-mass spectrometer) and Differential Scanning calorimetric analysis (METTLER TOLEDO STAR^e system at a heating rate of 10 °C min^{-1}, temperature range 30–350 °C using aluminum cans calibrated with indium) and elemental analysis (Perkin Elmer-2400 CHNO/S analyzer system). Solvent behavior of the compounds was studied by UV–Visible spectrophotometer (JASCO V-630 Spectrophotometer). The melting points were determined by open capillary method (Elico) and were uncorrected. The synthesized ligands were evaluated for their *in vitro* antimicrobial activity against different pathogens by Agar Well Diffusion method. The results of the potential antibacterial and analgesic activity of the selected ligands were rationalized by molecular docking.

The synthesis of the aryl/heteroaryl azo salicylic acid analogs was carried out on the basis of our earlier reported work [11] (Scheme 1).



Scheme 1 – 4-Benzenesulfoamido-(4a), 4-Sulfonic phenyl-(4b), 4-nitro phenyl-(4c), 4-methoxy phenyl-(4d), 4-bromo,3-methyl phenyl-(4e), 4-(1,5 dimethyl-2-phenyl)-pyrazol-3-one-(4f), 4-carboxy phenyl-(4g), N-(5-methylisoxazol-3-yl)benzene sulfonamide-(4h). Reactions: i.) $NaNO_2$/HCl, 0–5 °C, diazotization; ii.) 10% NaOH, coupling reaction.

Structures of some newly synthesized azosalicylic acid congeners.

2.1. *2-hydroxy-5-(4-sulfamoylphenylazo)-benzoic acid (4a)*

Dark red color powder; yield 75%; Rf 0.8; mp (°C); 297–300; UV–vis (λ_{max}, ethanol): 366 nm; IR (KBr, γ, cm^{-1}): 3374 (O—H str.), 1676 (C═O str.), 1587 (C═C str.), 1482 (—N═N—), 1331, 1160 (SO_2 str.SO_2NH_2), 910 (S-N str.), 1096 (C—O str.); ^{1}H NMR ($CDCl_3$, δppm, 400 *MHz*): 7.46 (s, 2H, $SO_2N\underline{H}_2$), 8.01–8.10 (m, 4H, Ar H), 12.10 (sb. 1H, COO<u>H</u>), 11.69 (sb, 1H, OH), 7.36 (d, 1H, salicylic H-3), 8.11 (d, 1H, salicylic H-4), 8.34 (s, 1H, salicylic H-6); LC–MS (% area); 77.65; *m/z*; 320.13 (M-1); Analysis for $C_{13}H_{11}N_3O_5S$: Calcd % C, 48.59; H, 3.45; N, 13.08; S, 9.98; Found %: C, 48.19; H, 3.48; N, 13.11; S, 9.95.

2.2. *2-hydroxy-5-(4-sulfamoylphenylazo)-benzoic acid (4b)*

Yellow color powder; yield 72%; Rf 0.8; mp (°C); 328–330; UV–vis (λ_{max}, ethanol): 361 nm; IR (KBr, γ, cm^{-1}): 3431 (O—H str.), 1671 (C═O str.), 1628 (C═C str.), 1448 (—N═N—), 1389, 1206 (SO_2 str.SO_3H), 1127 (C—O str.); ^{1}H NMR (DMSO-d_6, δppm, 400 *MHz*): 7.83–8.34 (m, 4H, Ar H), 11.69 (sb. 1H, O<u>H</u>), 12.10 (sb, 1H, COO<u>H</u>), 7.28 (d, 1H, salicylic H-3), 8.08 (d, 1H, salicylic H-4), 8.34 (s, 1H, salicylic H-6); LC–MS (% area); 52.33; *m/z*; 321.08 (M-1); Analysis for $C_{13}H_{10}N_2O_6S$: Calcd % C, 48.45; H, 3.13; N, 8.69; S, 9.95; Found %: C, 48.42; H, 3.09; N, 8.62; S, 9.91.

2.3. *2-hydroxy-5-(4-nitrophenylazo)-benzoic acid (4c)*

Dark red color powder; yield 92%; Rf 0.7; mp (°C); 243–245; UV–vis (λ_{max}, ethanol): 388 nm; IR (KBr, γ, cm^{-1}): 3456, 3210 (O—H str.), 1672 (C═O str.), 1610 (C═C str.), 1482 (—N═N—), 1530, 1344 (NO_2 str.), 1106 (C—O str.); ^{1}H NMR (DMSO-d_6, δppm, 400 *MHz*): 7.75–8.25 (m, 4H, Ar H), 11.75 (sb. 1H, O<u>H</u>), 12.09 (sb, 1H, COO<u>H</u>), 7.31 (d, 1H, salicylic H-3), 8.13 (d, 1H, salicylic H-4), 8.35 (s, 1H, salicylic H-6); LC–MS (% area); 91.62; *m/z*; 286.12 (M-1); Analysis for $C_{13}H_9N_3O_5$: Calcd % C, 54.36; H, 3.16; N, 14.63; Found % C, 54.26; H, 3.11; N, 14.60.