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SYNTHESIS, ANTIBACTERIAL AND ANTI-INFLAMMATORY ACTIVITIES OF SOME 2-PHENYLGLYOXYLIC ACID DERIVATIVES

A. OBAFEMI[1*], FESTUS O. TAIWO[1], EZEKIEL O. IWALEWA[2] AND DAVID A. AKINPELU[3]

[1]Department of Chemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.
.[2]Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.
[3]Department of Microbiology, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

ABSTRACT

Twelve phenylglyoxylic acid derivatives were synthesized, characterized and investigated for their antibacterial activity against nine Gram-positive and four Gram-negative bacterial isolates. Some of the synthesized compounds exhibited broad spectrum activity against the bacterial strains, with 2-[2-(acetylamino)phenyl]-N-(2-chlorophenyl)-2-oxoacetamide (9) being the most active, having MIC values of 7.8-1000 µg/ml and MBC of 31.3-2000 µg/ ml

Seven of the phenylglyoxylic acid derivatives were investigated for their anti-inflammatory activity (acute inflammation, pulmonary oedema and leucocyte counts) in mice. The reduction in oedema formation was between 22-67 % for all the synthesized compounds. The compounds showed significant anti-inflammatory activity with 2-[2-(acetylamino)phenyl]-N-(2,4-dichlorophenyl)-2-oxoacetamide (13) being the most active, while others appear variously dose-dependent.

Key words: Phenylglyoxylic acid amides, Synthesis, Antibacterial activity, Anti-inflammatory activity.

INTRODUCTION

Glyoxylic acid derivatives (esters and amides) and their simple analogs have been shown to be very useful in stereo-controlled organic synthesis, including natural products (Jurczak and Bauer, 2000) and possess therapeutically valuable properties (Nickel et. al., 2008). A novel synthesis of some n-alkyl-substituted 2,4-dihydroxyphenylglyoxylic acids have also been reported. The compounds were prepared via the condensation of ethyl cyanoformate with alkyl derivatives of 3-hydroxyphenol (resorcinol), using anhydrous HCl as catalyst, and screened for antimicrobial activity against three bacterial organisms (*Staphylococcus aureus, Bacillus subtilis* and *Escherichia coli)* and three fungi strains (*Epidermophyton floccosum, Microsporon canis* and *Tricophyton rubrum*). None of the compounds showed significant activity against *E. coli*, while only the sodium salt of 5-n-octyl-2,4-dihydroxyphenylglyoxylic acid showed activity against *S. aureus* (complete inhibition at 1:12,500 dilution), *B. subtilis* (complete inhibition at 1:25,000 dilution), *E. floccosum, M. canis and T. rubrum* (at 1: 8,000 dilution) (Hunsberger and Amstutz, 1948). Also, (3-Pyridyl)glyoxylic acid derivatives have been reported to exhibit antimicrobial properties (Nurullaeva et. al., 1986), while tropine phenylglyoxylate and its derivatives showed moderate cholinolytic activity (Glushkov et. al., 1977).

In addition, some derivatives of phenylglyoxylic acid (Figure 1), prepared from the reaction of α-ketocarboxylic acids with phenylhydrazine, semicarbazide, thiosemicarbazide and some alcohols, were established to possess various degrees of anti-inflammatory activity (Leont'eva et. al., 1993; Ismatov et. al., 2001).

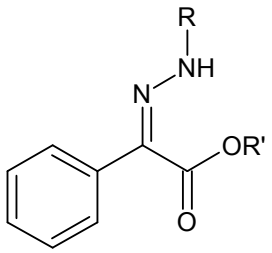


Figure 1. ***Phenylglyoxylic acid derivatives***

Inflammation is the body's natural protective response to tissue injury. The body tries to inactivate or destroy the invading microorganisms, remove irritants and thus set the stage for tissue repair (Madigan et. al., 2000). Microbial infections often cause inflammation and pain and in normal practice, analgesic, chemotherapeutic and anti-inflammatory drugs are prescribed simultaneously. It is uncommon to have a compound possessing the three activities.

In general, the cause of many diseases, such as periodontitis, is a combination of microorganisms and inflammatory response. For such diseases, compounds possessing dual antimicrobial and anti-inflammatory activities may be the appropriate therapeutic agents (Ishikawa, 2007). Only quite a few synthetic and natural compounds are known to exhibit both antibacterial and anti-inflammatory activities, including quercetin and trans-3,5,4'-trihydroxystilbene (resveratrol) (Cushnie and Lamb, 2005; Mahady and Pendland, 2000).

So far, the 2-aminophenylglyoxylic acid derivatives have not been studied for biological properties. Hence this study examines the antibacterial and anti-inflammatory properties of some 2-acetylaminophenylglyoxylic acid amides.

MATERIALS AND METHODS

CHEMISTRY: GENERAL

Melting points were determined with open capillary tubes on a Gallenkamp (variable heater) melting point apparatus (uncorrected). Infrared spectra were recorded as KBr pellets on a Buck spectrometer. ^{1}H and ^{13}C-NMR spectra were recorded at 400 MHz and 100 MHz, respectively, on a Bruker 400 MHz spectrometer (Botswana) and on a Varian 200 MHz spectrometer at the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife, Nigeria, using deuterated dimethylsulfoxide (DMSO-d_6), or $CDCl_3$ as solvent. The elemental analyses (C, H, N) of the compounds were performed using a Carlo Erba 1108 elemental analyzer and found to be within a range of ± 0.4 % of theoretical values. The purity of the synthesized compounds was checked by thin layer chromatography on silica gel plate, using $CHCl_3$: CH_3OH (9:1, v/v).

1-Acetyl-1H-indole-2, 3-dione (1)

Isatin (5.0 g, 34 mmol) was added to acetic anhydride (80 ml) and the mixture heated with continuous stirring at 90 – 100 ^{o}C for 3 h. The reaction mixture was allowed to cool and then put in a refrigerator to give fine yellow crystals of 1-acetylisatin, 1. The product was filtered and the mother liquor reduced to half its volume on a rotatory evaporator to get more of the product; Yield 5.3 g, 82 %)., m.p. 143 – 144 $^{\circ}$C. (Lit. (Pandey, (2010)), 137-139 ^{o}C). **IR** (cm^{-1}), 1770, 1750, 1715 (C=O), 1605 (C=C). **^{1}H-NMR** (δ,

ppm): 8.37 (d, 1H, C(7)-H), 7.30-7.73 (m, 3H, Ar-H), 2.70 (s, 3H, CH_3). **^{13}C NMR** (50 MHz, DMSO-d6): δ 180.1 (C=O), 169.5 (C=O), 157.8 (C=O), 148.5 (Q), 138.7 (CH), 126,1 (CH), 125.1(CH), 119.1 (Q), 118.2 (CH), 26.2 (CH_3).

2-[2-(acetylamino)phenyl]-2-oxo-N-(4-sufamoylphenyl)acetamide (2)

A mixture of 1-acetylisatin 1 (1.0 g, 5.3 mmol.) and 4-aminobenzenesulfonamide (0.91 g, 5.3 mmol.) and potassium carbonate (0.88 g, 1.2 mol. equivalent) in acetonitrile (30 ml)
was stirred at room temperature for 8 h. The precipitate formed was filtered to give 2 as yellow crystals (0.67 g, 41 %), m.p. 201-203 ˚C. **IR** (cm^{-1}): 3478 (NH), 3381 (NH), 3277 (NH), 1666 (C=O), 1593 (C=C), 1313, 1143 (SO_2). **^{1}H-NMR** (DMSO-d_6): 10.51 (s, br, 1H, NH, D_2O exchangeable), 7.90 (d, 2H, ArH), 7.85 (m, 4H, ArH), 7.39 (m, 2H, SO_2NH_2, D_2O exchangeable), 7.16 (d, 2H, ArH), 2.34 (s, 3H, CH_3). **Anal. Calcd**. for $C_{16}H_{15}N_3O_5S$ (361.37): C, 53.18; H, 4.18; N, 11.63. Found: C, 53.35; H, 4.05; N, 11.54; %.

Compounds 3-13 were prepared in a similar manner:

2-[2-(acetylamino)phenyl-2-oxo-N-phenylacetamide (3)

Obtained as white solid (yield, 90 %), m.p. 197-199 ^{o}C (dec).. **IR** (cm^{-1}): 3448 (NH), 3265 (NH), 1621 (C=O), 1532, 1380, 1301, 1216. **^{1}H-NMR** ($CDCl_3$): 10.91 (s, 1H, NH, D_2O exchangeable), 8.97 (s, br, 1H, NH, D_2O exchangeable), 8.61 (d, 1H, ArH), 8.43 (d, 1H, ArH), 7.71 (m, 2H, ArH), 7.59 (t, 1H, ArH), 7.42 (m, 2H, ArH), 7.25 (m, 1H, ArH), 7.16 (t, 1H, ArH), 2.24 (s, 3H, CH_3). **Anal. Calcd**. for $C_{16}H_{14}N_2O_3$ (282.29): C, 68.07; H, 5.00; N, 9.92. Found: C, 67.88; H, 4.97; N, 9.99; %.

2-[2-(acetylamino)phenyl]-N-(4-acetylphenyl)-2-oxo-acetamide (4)

Yellow crystals (yield, 98.5 %), m.p. 299-301 ˚C; **IR** (cm^{-1}): 3374 (NH) 3265 (NH) 1699 (C=O) 1599, 1380, 1301, 1216.

^{13}C- NMR: (CDCl3): 191.2 (C=O), 169.4 (C=O), 160.2 (C=O), 142.1, 136.7, 134.4, 129.3, 125.5, 122.7, 120.8, 120.1, 118.7, 25.0**. Anal. Calcd.** for $C_{18}H_{16}N_2O_4$ (324.33): C, 66.66; H, 4.97; N, 8.64. Found: C, 66.60; H, 5.00; N, 8.60; %.

2-[2-(acetylamino)phenyl]-2-oxo-N-{4-(phenyldiazenyl) phenyl} acetamide (5)

Orange crystals (yield, 82.8 %). m.p. 206-208 ˚C ; **IR** (cm^{-1}): 3283 (NH), 3277 (NH), 1670 (C=O), 1605 (C=O, C=C), 1405, 1320, 1216**. ^{1}H-NMR** ($CDCl_3$): 10.85 (s, 1H, NH, D_2O exchangeable), 8.97 (s, 1H, NH, D_2O exchangeable), 8.69 (d, 1H, ArH) , 8.60 (d, 1H, ArH), 8.0 (m, 2H, ArH), 7.92 (m, 4H, ArH), 7.68 (t, 1H, ArH), 7.53 (m, 3H, ArH), 7.24 (t, 1H, ArH), 2.29 (s, 3H, $COCH_3$)**. ^{13}C- NMR:** 190.2 (C=O), 169.2 (C=O), 159.6 (C=O), 152.7, 149.8, 142.3, 138.8, 136.9, 134.5, 131.0, 129.1, 124.2, 122.9, 122.7, 122.2, 121.0, 120.2, 119.9, 118.8, 25.5. **Anal. Calcd.** for $C_{22}H_{18}N_4O_3$ (386.40): C, 68.38; H, 4.70; N, 14.50. Found: C, 68.40; H, 4.74; N, 14.55; %.

2-[2-acetylamino)phenyl]-N-[(5-methyl-1,2-oxazol-3-yl)sulfamoyl]phenyl}-2-oxoacetamide (6)

A mixture of 1-acetylisatin 1 (1.0 g, 5.3 mmol) and septrin (1.36 g, 5.3 mmol) and potassium carbonate (0.87 g, 1.2 mol equivalent) in acetonitrile (50 ml) was stirred at room temperature for 2 h. The precipitate formed was filtered to give 6 as white solid mixture (1.5 g, 90 %) m.p. >340 ^{o}C; **IR** (cm^{-1}): 3441 (NH), 3259 (NH), 1619 (C=O), 1532, 1301, 1161. **^{1}H-NMR** ($CDCl_3$): 7.87 (s, br, 2H, NH, D_2O exchangeable), 7.64 (d, 2H, ArH), 6.64 (d, 2H, ArH), 6.41 (s, 2H, ArH), 6.22 (s, 1H, ArH), 5.89 (s, NH, D_2O exchangeable), 3.84, 3.85 (s, 6H, OCH_3), 3.68 (s, 2H), 2.36 (s, 3H, CH_3).

N-[2-{Oxo(piperidin-1-yl)acetyl]phenyl}acetamide (7)

Yellow solid (yield, 63.9 %); m.p. >300 ^{0}C; **IR** (cm^{-1}): 3399 (NH), 1691 (C=O), 1630, 1605, 1405, 1320, 1216**; ^{1}H-NMR** ($CDCl_3$): 11.33 (s, 1H, NH, D_2O exchangeable), 8.81 (d, 1H, ArH), 7.64 (m, 2H, ArH), 7.17 (t, 1H, ArH), 3.72 (m, 2H), 3.30 (m, 2H) 2.29 (s, 3H, $COCH_3$), 1.73 (m, 4H, $2CH_2$), 1.58 (s, br, 2H, CH_2). **^{13}C-NMR** : 196.5 (C=O), 169.5 (C=O), 164.4 (C=O), 142.4, 136.8, 133.5,

122.7, 120.7, 118.0, 47.2, 42.2, 26.1, 25.5, 25.4, 24.3. **Anal. Calcd.** for $C_{15}H_{18}N_2O_3$ (274.31): C, 65.68; H, 6.61; N, 10.21. Found: C, 65.52; H, 6.64; N, 10.13; %.

2-[2-(acetylamino)phenyl]-N-(4-methylphenyl)-2-oxoacetamide (8)

Yellow crystals, (yield, 90.7 %). m.p. 310-312 ^{o} C . **IR** (cm^{-1}): 3393 (NH), 1688 (C=O), 1650 (C=O), 1618, 1295,1156. **^{1}H-NMR** ($CDCl_3$,): 10.88 (s, br, 1H, NH, D_2O exchangeable), 8.69(d, 1H, ArH,), 8.66 (s, br, 1H, NH, D_2O exchangeable), 8.58 (dd, 1H, ArH), 7.67 (t, 1H, ArH), 7.59 (m, 2H, ArH), 7.22 (m, 3H, ArH), 2.39 (s, 3H, Ar-CH_3), 2.28 (s, 3H, $COCH_3$), **^{13}C NMR :** 191.0 (C=O), 169.2 (C=O), 160.0 (C=O), 142.1, 136.7, 135.4, 134.5, 134.0, 129.8, 122.6, 120.9, 120.0, 118.9, 25.4, 21.0. **Anal.Calcd**. for $C_{17}H_{16}N_2O_3$ (296.32): C, 68.91; H, 5.44; N, 9.45. Found: 68.81; H, 5.46; N, 9.41; %.

2[2-(acetylamino)phenyl]-N-(2-chlorophenyl)-2-oxo- acetamide (9)

Brown solid (yield, 89.5 %). m.p. 304-306 ^{o}C; **IR** (cm^{-1}) : 3472 (NH), 3302 (NH), 1697 (C=O), 1599 (C=O, C=C), 1374, 1228. **^{1}H-NMR** ($CDCl_3$): 10.82 (s, 1H, NH, D_2O exchangeable), 7.17-8.50 (m, 7H, ArH + 1H, NH), 8.75 (m, 1H, ArH), 2.31 (s, 3H, $COCH_3$). **Anal. Calcd.** for $C_{16}H_{13}ClN_2O_3$ (316.74): C, 60.67; H, 4.14; N, 8.84. Found: C, 60.50; H, 4.10; N, 8.79; %.

2-[2-(acetylamino)phenyl]-N-(4-methoxylphenyl)-2-oxo-acetamide (10)

White crystals, (yield, 90 %). M.p. 296-297 ^{o} C; **IR** (cm^{-1}) : 3259 (NH), 3144 (NH), 1684 (C=O), 1654 (C=O), 1605,1374,1289. **^{1}H-NMR** ($CDCl_3$): 10.90 (s, 1H, NH, D_2O exchangeable), 8.73 (s, br, 1H, NH, D_2O exchangeable), 8.66 (d, 1H, ArH), 8.54 (d, 1H, ArH), 7.63 (m, 3H, ArH), 7.17 (t, 1H, ArH), 6.95 (m, 2H, ArH), 3.85 (s, 3H, OCH_3), 2.26 (s, 3H, $COCH_3$). **^{13}C-NMR:** 191.2 (C=O), 169.2 (C=O), 159.7 (C=O), 157.3 , 142.1, 136.6, 134.4, 129.7, 122.6, 121.7, 120.8, 118.9, 114.4, 55.5, 25.4. **Anal. Calcd.** for $C_{17}H_{16}N_2O_4$ (312.32): C, 65.38; H, 5.16; N, 8.97. Found: C, 65.33; H, 5.11; N, 9.06; %.

2-[2-acetylamino) phenyl]-N-(2-aminophenyl)-2-oxo-acetamide (11)

White solid, (yield, 94.5 %). m.p. 203-205 ^{o} C; **IR** (cm^{-1}): 3397 (NH), 1684 (C=O), 1599 (C=C), 1417, 1228, 1168. **Anal. Calcd.** for $C_{16}H_{15}N_3O_3$ (297.31): C, 64.64; H, 5.09; N, 14.13. Found: C, 65.03; H, 4.79; N, 14.50; %.

2-[2-(acetylamino)phenyl]-N-(2-ethoxyphenyl)-2-oxo-acetamide (12).

Yellow crystals, (yield, 71.6 %), m.p. 148-150 ^{o} C; **IR** (cm^{-1}): 3289 (NH), 1709 (C=O), 1642 (C=O), 1259, 1169, 1119. **^{1}H-NMR** ($CDCl_3$): 11.03 (br, s, 1H, NH, D_2O exchangeable), 9.53 (s, 1H, NH, D_2O exchangeable), 9.18 (m, 1H, ArH), 8.82 (m, 1H, ArH), 7.64 (m, 2H, ArH), 7.14-7.20 (m, 2H, ArH), 6.91-7.04 (m, 2H, ArH), 4.13 (q, 2H, CH_2), 2.24 (s, 3H, $COCH_3$), 1.44 (t, 3H, CH_3). **Anal. Calcd**. for $C_{18}H_{18}N_2O_4$ (326.35): C, 66.25; H, 5.56; N, 8.58. Found: C, 66.28; H, 5.50; N, 8.60; %.

2-[2-(acetylamino)phenyl]-N-(2, 4-dichlorophenyl)-2-oxoacetamide (13)

Brown crystals (yield, 97.7 %). m.p. 308-310 ^{0}C; **IR** (cm^{-1}): 3269 (NH), 1666 (C=O), 1636 (C=O), 1593 (C=C), 1313, 1119**. ^{1}H-NMR:** 10.98 (s, 1H, NH, D_2O exchangeable), 8.79 (m, 1H, ArH), 8.09 (d, J = 8.70 Hz, 1H, ArH), 7.78 (d, J = 2.26 Hz, 1H, ArH), 7.66-7.56 (m, 3H, ArH), 7.18 (dd, 1H, ArH), 2.23 (s, 3H, $COCH_3$). **Anal. Calcd.** for $C_{16}H_{12}Cl_2N_2O_3$ (351.18): C, 54.72; H, 3.44; N, 7.98. Found: C, 54.56; H, 3.46; N, 8.07; %.

ANTIMICROBIAL SCREENING MICROORGANISMS

The following standard bacteria of National Collection for Industrial Bacteria (NCIB) and Locally Isolated Organisms (LIO) used in this research work were obtained from the culture collection of Dr. D.A Akinpelu of the Department of Microbiology, Obafemi Awolowo University, Ile- Ife, Osun state, Nigeria:

Bacillus cereus (NCIB 6349), *Bacillus polymyxa* (LIO), *Bacillus stearothermophilus* (NCIB 8222), *Clostridium sporogenes* (NCIB 532),

Bacillus substilis (NCIB 3610), *Corynebacterium pyogenes* (LIO), *Escherichia coli* (NCIB 86), *Klebsiella pnemoniae* (NCIB 418), *Pseudomonas aeruginosa* (NCIB 950), *Pseudomonas fluorescens* (NCIB 3756), *Enterococcus faecalis* (NCIB 755), *Staphylococcus aureus* (NCIB 8588), *Staphylococus epidermidis* (LIO).

ANTIBACTERIAL SENSITIVITY TESTING OF THE COMPOUNDS, 2-13

All the synthesized compounds were screened for antibacterial activity using the agar well diffusion method as described by Akinpelu (Akinpelu, 1999). The medium employed was diagnostic sensitivity test agar (Biotech .Ltd).

With the aid of a sterile 1 ml pipette, about 0.2 ml of the broth culture of test organism was added to 18 ml sterile molten diagonist sensitivity test agar (Biotech Ltd) which had already cooled down to 45 ˚C. This was well mixed and poured into previously sterilized Petri dishes, which have been properly labeled according to the test organism. The medium was then allowed to set. With the aid of a sterile cork borer, the required numbers of holes were bored into the medium.

The wells were made of about 5 mm to the edge of the plate. The wells were filled up aseptically with the solution of the compound using Pasteur pipettes. Streptomycin phosphate was used as the standard antibacterial agent at a concentration of 1 mg/ml. The plates were allowed to stand for about one hour on the bench to allow for proper diffusion of antibacterial agent into the medium and then incubated uprightly at 37 ˚C for 24 hours. Care was taken not to stockpile the plates. Clear zones of inhibition indicated the relative susceptivity of the bacteria to the compounds. These were recorded in millimeters.

DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The minimum inhibitory concentration (MIC) was determined using the method of Russell and Furr (Russell and Furr, 1977). Different concentrations of the compounds were prepared using a two-fold dilution method in DMSO solvent. The concentration ranged between 0.0078 and 1.000 mg/ml. About 2 ml of the solution of each compound from each dilution was put into a sterile plate with the aid of sterile pipette and then mixed with 18 ml of molten Nutrient agar. This was then allowed to set. The surface of the nutrient agar plate was allowed to dry before streaking with overnight broth cultures of the bacterial isolates. The plates were then labeled accordingly and incubated at 37 ˚C for 72 hours. They were subsequently examined for the presence or absence of growth. The lowest concentration preventing growth was taken as the minimum inhibitory concentration of the compound. This procedure was employed for all the compounds.

DETERMINATION OF MINIMUM BACTERICIDAL CONCENTRATIONS (MBCS).

The minimum bactericidal concentrations of the compounds were determined as described in the literature (Olorundare et. al., 1992) with little modifications. Samples were taken from plates with no visible growth in the MIC assay and subcultured onto freshly prepared nutrient agar medium and later incubated at 37 ^{o}C for 48 h. The MBC was taken as the lowest concentration of the compound that completely inhibited bacterial growth.

ANTI-INFLAMMATORY SCREENING ANIMALS

Albino mice of both sexes (18 – 27 g) (Vom strain; National Veterinary Research Institute, Vom, Nigeria) were used. All the animals were bred and housed in well lit and aerated room in the Animal House, Department of Pharmacology, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, according to standard animal care protocols. All animals had free access to drinking water and standard commercial diet (Guinea Feeds Brand, Bendel Feeds and Flour Mills Ltd, Ewu, Nigeria). The “principle of laboratory animal care” (NIH publication No 85-23) guidelines and procedures were followed in this study (NIH publication revised, 1985).

Drugs and Chemicals (Analar grade)

The drugs used for the experiments in this work are: Carrageenin (BDH Chemical Ltd, England), Indomethacin (KGN Pharmaceuticals), Dimethyl sulfoxide (DMSO) (Sigma), sodium chloride

(BDH). All drugs were administered as a solution of normal saline on each day of the experiment.

ANTI-INFLAMMATORY ACTIVITY ACUTE INFLAMMATION

Carrageenin-induced paw oedema in mice was used as a model of acute inflammation. 0.1 ml of a 1% carrageenin solution was injected into the plantar surface of the right hind paws of the mice. Control group mice (n = 5) received normal saline (0.3 ml/kg i.p) treatment only, while animals in the test group were treated with compounds 4, 5, 6, 7, 10, 11, and 13 (50, 100, 200 & 400 mg/kg i.p).or Indomethacin (10 mg/kg i.p) 1 hour before carrageenin injection. 2 hours after carrageenin injection, the mice were anaesthetized by dropping them in a jar containing cotton wool soaked with chloroform, and both the right and left hind limbs were cut identically at the ankle joint and weighed. The differences in weight gave the amount of oedema developed in the right hind limbs (Subramoniam et. al., 2001).

CARRAGEENIN-INDUCED PLEURISY IN MICE

The method used in these experiments was modified from that described in detail earlier (Vinegar et. al., 1982), cited by Badilla *et al* (2003). 5 groups of mice each, were treated with 4, 5, 6, 7, 10, 11, and 13 (50, 100, 200 & 400 mg/kg i.p), IND (10 mg/kg i.p) and normal saline [control] (2 ml/kg i.p), respectively. One hour after treatment, all the animals received an intrapleural injection of 0.1 ml carrageenin on the right side of the thorax. 2 hours later, the mice were anaesthetized with chloroform, and the pleural cavity was washed with.0.1 ml of distilled water. The number of leucocytes in the pleural cavity was determined and recorded.

PULMONARY OEDEMA

The lungs of the animal sacrificed above were dissected free from the trachea and weighed. Significant changes in the test "wet-lung weight" compared to the distilled water-treated controls, were considered to reflect pulmonary oedema (Staub, 1974).

Pulmonary oedema was calculated from the formula:

$$\text{Pulmonary oedema} = \frac{\text{Lungs wet weight}}{\text{Body Weight}} \times \frac{10{,}000}{1}$$

STATISTICAL ANALYSIS OF RESULTS

Results of the experiments and observations are expressed as mean ± standard error of mean (s.e.m) in this report. The significance of differences between groups was determined using one-way analysis of variance (ANOVA), followed by post hoc analysis using the Student-Newman-Keuls test. The results were validated by a computer programme developed by the United States Environmental Protection Agency (USEPA) Version 1.5. In all the observations, statistical significance was accepted at p values less than or equal to 0.10, 0.001 and 0.005. In all these statistical determinations, a computer programme-the Primer of Biostatistics (Version 3.01) was used.

RESULTS AND DISCUSSION

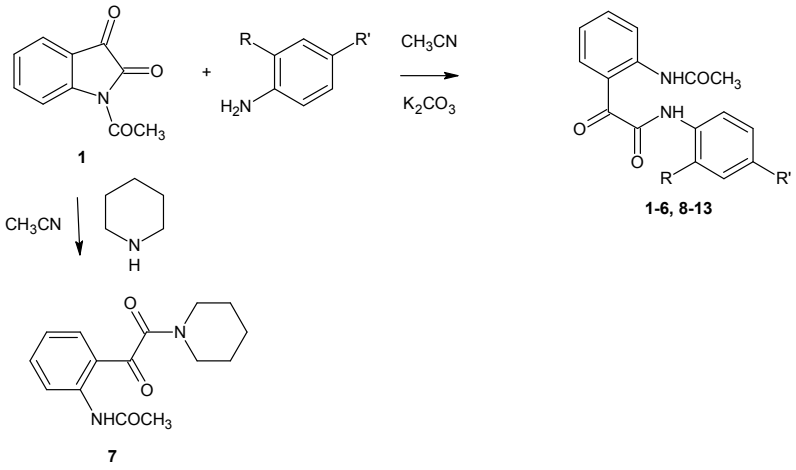
Synthesis of the compounds

Isatin and its derivatives having electron-withdrawing groups on nitrogen, such as 1-acetyl-1H-indole-2,3-dione (**1**), have been shown to react with nucleophiles (such as amines and alcohols) with concomitant ring-opening at the Nitrogen-Carbon-2 bond to give phenylglyoxylic acid derivatives (Bergman et. al., 1976).

1-acetyl-1H-indole-2,3-dione **1** was prepared by the reaction of isatin with acetic anhydride. The phenylglyoxylic acid derivatives (**2** - **13**) were prepared from the reaction of 1-acetylisatin **1** with the appropriate anilines in acetonitrile. For example,

N-[2-[oxo(piperidin-1-yl)acetyl]phenyl}acetamide, **7**, was prepared from the reaction of **1** with piperidine, while 2-[2-acetylamino)phenyl]-N-[4-[(5-methyl-1,2-oxazol-3-yl)sulfamoyl]phenyl}-2-oxoacetamide (**6**) was prepared from the reaction of **1** with septrin (Figure 2).

In the ^{1}H nmr, the acetylamino NH signal showed in the range δ 10.51-11.30, while the other NH showed in the range δ 7.39-9.00. In the ^{13}C nmr, the C=O groups showed in the ranges 190.2-196.5, 169.2-169.5 and 159.6-164.4 ppm.



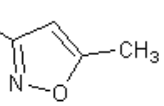
Compound No	R	R'
2	H	SO_2NH_2
3	H	H
4	H	$COCH_3$
5	**H**	**-N=N-Ph**
		O_2SHN  CH_3
6	H	
8	H	$-CH_3$
9	Cl	H
10	H	OCH_3
11	$-NH_2$	H
12	OCH_2CH_3	H
13	Cl	Cl

Figure 2: ***Synthetic route to Compounds 2-13***

Antimicrobial activity

The synthesized compounds, **2-13** were screened in-vitro for possible antibacterial activity. The sensitivity patterns of zones of inhibition (in mm) exhibited by the synthesized compounds (at 2 mg/ml), streptomycin and tetracycline (two reference clinical antibiotics, at 1 mg/ml each) and DMSO (solvent) against nine species of Gram-positive and four Gram- negative bacteria are reported in Tables 1 and 2. In general, the results showed that all the synthesized compounds, 2-13 exhibited broad spectrum activity against the bacterial strains.

Compound **4** showed activity against all the nine Gram-positive bacterial strains as was observed for streptomycin (although *Staph*. *aureus* was resistant

to tetracycline), while compounds **3**, **9** and **10** showed activity against seven of the nine microorganisms. However, compound 4 showed larger zones of inhibition than streptomycin for all the strains except *B. stearothermophillus*, *B. subtilis* and *Staph*. *aureus* and larger zones of inhibition than tetracycline on six of the strains. All the compounds exhibited antibacterial activity against *B. stearothermophillus* (except compound **6**) and *B. subtilis*, with zones of inhibition ranging from 15 mm to 27 mm. Only six of the compounds showed varying zones of inhibition (10 – 16 mm) against *Staphylococcus aureus* .

Table 1 ***Results of the antibacterial screening (sensitivity testing) of the 2-phenyl-2-oxoacetamide derivatives on Gram-positive bacteria with the zone of inhibition in mm (at 2 mg/ml).***

Compound No→ Microorganisms↓	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**STREP (1 mg/ml)**	**TET (1 mg/ml)**
Staphylococcus epidermidis (LIO)	0	14	32	0	0	0	0	38	12	0	16	10	18	10
Bacillus polymyxa (LIO)	0	0	18	10	0	15	12	8	0	10	0	0	15	20
Bacillus cereus (NCIB 6349)	0	15	32	18	0	20	22	0	14	13	12	16	28	18
Enterococcus faecalis (NCIB 775)	36	12	28	14	20	14	12	40	10	0	0	17	23	28
Corynobacterium pyogenes (LIO)	15	0	37	0	22	0	0	10	0	0	0	0	20	20
Clostridium sporogenes (NCIB 532)	20	16	32	0	15	0	14	30	13	15	12	12	25	20
Bacillus stearothermophillus (NCIB 8222)	20	20	23	25	0	27	25	22	18	20	21	20	23	22
Bacillus subtilis (NCIB 3610)	18	22	20	20	18	18	15	26	17	18	16	22	20	22
Staphylococcus aureus (NCIB 8588)	16	14	12	0	0	0	0	0	12	15	10	0	21	0

- ***Strep = Streptomycin***
- ***LIO = Locally Isolated Organism***
- ***NCIB = National Collection of Industrial Bacteria***
- ***TET = Tetracycline***

On the other hand, for the Gram-negative bacterial strains (Table 2), all the compounds and tetracycline showed activity against *Escherichia coli,* with zones of inhibition ranging from 18 - 28 mm, with compound **7** showing the largest zone of inhibition (28 mm). Only three of the compounds (**4**, **9** and **10**) exhibited activity against the four Gram-negative bacterial isolates with compound **9** showing the largest zone of inhibition. It is noteworthy that the antibiotic standard, streptomycin, showed no activity against *E. coli* and *K. pneumoniae*, while tetracycline showed no activity against *Ps. fluorescens* and *Ps. aeruginosa*.

The lowest concentrations of drug that completely inhibited the growth of organism (MIC values) for some selected compounds are shown in Tables 3 and 4. The compounds were selected based on their large zones of inhibition and broad spectrum of activity. The MICs of compounds **4**, **9** and **10** (for Gram-positive bacterial isolates) varied between 7.8 and 1000 µg/ml, while the MIC values for streptomycin varied between 7.8 and 500 µg/ml. Compound **4** showed its highest activity against *C. pyogenes* and *B. subtilis* with MIC value of 15.7 µg/ml, while compound **9** exhibited its highest activity against *E. faecalis* with MIC of 7.8 µg/ml. Compound **10** gave an MIC value of 7.8 µg/ml against *B. cereus*. The result indicated that compound **9** has better activity than the standard streptomycin on Gram positive organisms: *S. epidermidis*, *B. polymyxa*, *E. faecalis* and *C. pyogenes*.

The MIC value for the three compounds (**4**, **9** and **10**) is 1000 µg/ml for Gram-negative bacterial strains, *E. coli* and *K. pneumoniae* (for which streptomycin showed no sensitivity) (Table 4).

The minimum bactericidal concentrations (MBCs) exhibited by the test compounds against some selected bacterial isolates are as indicated in Tables 5 and 6. The MBC of compounds **4**, **9** and **10** against Gram positive bacteria ranged between 31.3 and 2000 µg/ml, while that of the standard antibiotic, streptomycin, varied between 15.7 and 1000 µg/ml. The results indicated that streptomycin has stronger activity than the test compounds for some of the bacterial strains as shown in Table 5. In comparison, the MBC exhibited by compound 4 against *Coryn. pyogenes* and *B. subtilis* (31.3 µg/ml) is stronger than the one shown by streptomycin (which are 125 µg/ml for *Coryn. pyogenes* and 250 µg/ml for *B. subtilis*). Compound **9** also showed similar stronger activity against *E. faecalis* and *Coryn. pyogenes* (31.3 µg/ml) when compared with the activity exhibited by streptomycin against the same organisms (125 µg/ml).

The MBC exhibited by compounds **4**, **9** and **10** against the three Gram negative bacterial isolates are shown in Table 6. The three test compounds showed weak MBC against the Gram negative bacterial isolates when compared with the activities exhibited by the compounds against Gram positive isolates. Gram negative bacteria are known to be inherently more resistant to antimicrobial compounds than Gram positive bacteria. The resistant capability of Gram negative bacteria to antimicrobial compounds is associated with their cell-wall which is made up of cytoplasmic membrane containing lipids and protein, a thin peptidoglycan layer and an outer membrane composed of lipopolysaccharide Pelczar et. al., 2006).

Table 2. ***Result of the antimicrobial screening (sensitivity testing) on Gram-negative bacteria with the zone of inhibition in mm (at 2 mg/ml).***

Compound No → microorganismss↓	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	***Strep (1 mg/ml)**	**TET (1 mg/ml)**
Escherichia coli (NCIB 86)	22	25	22	24	20	28	25	19	18	20	21	21	0	18
Pseudomonas fluorescens (NCIB 3756)	10	10	20	0	0	0	0	30	13	15	12	12	30	0
Klebsiella pneumoniae (NCIB 418)	0	0	22	0	0	0	0	32	12	0	0	0	0	12
Pseudomonas aeruginosa (NCIB 950)	0	16	17	0	0	0	0	20	12	12	12	14	22	0

**** Strep = Streptomycin***

TET = Tetracycline. NCIB = National Coolection of Industrial Bacteria

Table 3 ***Minimum inhibitory concentration (MIC) for some selected compounds (mg/ml) on various Gram positive bacteria***

Compound No→ Microorganisms↓	**4**	**9**	**10**	***Strep**
Staphylococcus epidermidis (LIO)	0.0313	0.0157	1.000	0.0313
Bacillus polymyxa (LIO)	1.000	0.0157	NA	0.125
Bacillus cereus (NCIB 6349)	ND	NA	0.0078	0.0313
Enterococcus faecalis NCIB 775)	1.000	0.0078	1.000	0.0625
Corynebacterium pyogenes (LIO)	0.0157	0.0157	NA	0.0313
Clostridium sporogenes (NCIB 532)	0.0625	0.0157	1.000	0.0078
Bacillus stearothermophilus (NCIB 8222)	1.000	0.250	0.250	0.0625
Bacillus subtilis (NCIB 3610)	0.0157	1.000	1.000	0.0625
Staphylococcus aureus (NCIB 8588)	1.000	NA	1.000	0.500

**** Strep = Streptomycin; NA = Not active***

Table 4 ***Minimum inhibitory concentration for some selected compounds in (mg/ml) on various Gram negative bacteria***

Compound No→ Microoganisms↓	**4**	**9**	**10**	**Strep**
Escherichia coli (NCIB 86)	1.000	1.000	1.000	NA
Pseudomonas aeruginosa (NCIB 950)	1.000	1.000	1.000	0.2500
Klebsiella pneumoniae (NCIB 418)	1.000	1.000	1.000	NA

**** Strep = Streptomycin; NA = Not Active***

Table 5. ***Minimum bactericidal concentration (MBC) for some selected compounds (mg/ml) on various Gram positive bacteria.***

Compound No→ Microorganisms↓	**4**	**9**	**10**	*Strep
Staphylococcus epidermidis (LIO)	0.0625	0.0313	2.000	0.0625
Bacillus polymyxa (LIO)	2.000	0.0625	NA	0.500
Bacillus cereus (NCIB 6349)	NA	NA	0.0313	0.0625

Enterococcus faecalis NCIB 775)	2.000	0.0313	2.000	0.125
Corynebacterium pyogenes (LIO)	0.0313	0.0313	NA	0.125
Clostridium sporogenes (NCIB 532)	0.125	0.0313	2.000	0.0157
Bacillus stearothermophilus (NCIB 8222)	2.000	0.500	0.500	0.250
Bacillus subtilis (NCIB 3610)	0.0313	2.000	2.000	0.250
Staphylococcus aureus (NCIB 8588)	2.000	NA	2.000	1.000

**** Strep = Streptomycin; NA = Not Active***

Table 6. ***Minimum bactericidal concentration (MBC) for Some selected compounds in (mg/ml) on various Gram negative bacteria***

Compound No→ Microoganisms↓	**4**	**9**	**10**	***Strep**
Escherichia coli (NCIB 86)	2.000	2.000	2.000	NA
Pseudomonas aeruginosa (NCIB 950)	2.000	2.000	2.000	1.000
Klebsiella pneumoniae (NCIB 418)	2.000	2.000	2.000	NA

**** Strep = Streptomycin; NA = Not Active***

Anti-inflammatory Activity

Three different models of determining anti-inflammatory activity were used to screen compounds **4**, **5**, **6**, **7**, **10**, **11** and **13** at four different doses (50, 100, 200 and 400 mg/kg) and the results are shown in Table 7. The activity shown in mice right hind paw (oedema) for compounds **4**, **5**, **6**, and **11** (at 100 mg/ml) were better than the standard drug, indomethacin (at 10mg/ml).

The activity shown by all the tested compounds in pulmonary oedema formation were better than the standard drug indomethacin at 50 mg/ml. Effects were also demonstrated against leucocytes by the compounds, with compounds **11** and **13** exhibiting better activity than indomethacin at 100 mg/ml. However, compounds **4**, **5**, **6**, and **7** exhibited less activity than indomethacin. The reduction in oedema formation weight (Table 7) was between 22.2-44.4 % at 50 mg/ml dose, while at 100 mg/ml dose, the % inhibition was between 31.1-55.6 %, compared to indomethacin which gave 36.70 %. It is interesting to note that all the compounds showed significant activity using the three different models of determining the anti-inflammatory properties of chemical compounds. It should also be noted that all the compounds exhibited dose-related inhibition of leucocytes counts where the anti-inflammatory property was well demonstrated. The order of

activity for leucocyte count and pulmonary oedema (at 100 mg/ml) is **13** >**11** > **10** > **6** > **7** > **4** > **5** for all the compounds.

Pain and swelling (oedema) are among the signs and symptoms of both acute and chronic inflammatory and stress. Indomethacin and other non-steroidal anti-inflammatory drugs (NSAIDs) are prominent agents used for or in combination with agents used in treating these symptoms. The results of this investigation showed that all the tested compounds produced a significant anti-inflammatory action.

Table 7: ***Anti-Inflammatory activity of some 2-phenyl-2-oxo-acetamide derivatives.***

Dosage mg/kg Drug	Oedema formation	Inhibition %	Pulmonary Oedema	Number of Leucocytes
Control (Distilled Water 2ml/kg i.p) with carrageenin	0.09±0.03	0	112.45±1.8	197.22±20.88
4				
50	0.07±0.01	22.2±0.04	56.43±16.23**	85.24±5.96**
100	0.05±0.01	44.4±0.04	48.92±11.21**	82.12±1205**
200	0.04±0.01*	55.6±0.04	48.92±11.21**	82.12±1205**
400	0.04±0.02*	55.6±0.05	38.60±5.35**	77.12±11.59**
5				
50	0.06±0.01*	33.3±0.04	73.00±11.01	176.44±5.8*
100	0.05±0.01	44.4±0.04	65.96±11.90**	94.88±16.07**
200	0.05±0.02*	44.4±0.05	60.67±9.8**	84.00±19.22**
400	0.04±0.02*	55.6±0.05	51.90±5.29**	70.56±17.06**
6				
50	0.06±0.01	33.3±0.04	60.61±3.013	161.90±25.88**
100	0.05±0.01	33.3±0.05	59.33±8.81**	110.76±11.11**
200	0.04±0.01*	55.6±0.04	53.57±6.21**	102.76±9.89**
400	0.03±0.01**	66.7±0.04	55.07±11.57**	99.76±16.52**
7				
50	0.06±0.01	33.3±0.04	70.35±7.21**	107.70±5.02**
100	0.06±0.02	33.3±0.05	70.42±10.37**	96.52±27.37**
200	0.05±0.02	44.4±0.05	60.15±8.21**	90.80±18.57**
400	0.04±0.02	55.6±0.05	50.49±2.10**	65.52±14.15
10				
50	0.06±0.01	33.3±0.04	74.30±13.22**	107.26±3.18**
100	0.062±0.02	31.11±0.05	71.94±13.27**	60.92±6.55***
200	0.04±0.02*	55.6±0.05	76.93±3.14**	69.16±14.12**
400	0.03±0.01**	66.7±0.05	7.99±7.29**	56.36±153.36**

11				
50	0.05±0.01	44.4±0.04	76.30±3.94**	112.22±10.15*
100	0.04±0.01*	55.6±0.04	65.82±9.51**	31.30±2.98**
200	0.04±0.02*	55.6±0.05	69.46±16.91**	30.88±2.31
400	0.03±0.01**	66.7±0.04	56.19±5.47**	32.36±9.27**
13				
50	0.07±0.01	22.2±0.04	69.57±1.60*	126.00±27.88**
100	0.06±0.02	33.3±0.05	75.01±7.75**	29.00±5.59**
200	0.04±0.02*	55.6±0.05	75.11±7.46**	29.76±3.36
400	0.04±0.03**	55.6±0.07	90.36±38.55**	27.36±4.38**
Indomethacin (10 mg/kg)	0.038±0.005	36.70	83.3±2.40**	49.12±0.80

P<0.10; ** P< 0.05; *** P<0.001

Values given report the mean ±S.E.M of five observations.

Although the present study could not establish the exact mechanism of the anti-inflammatory actions of the synthesized compounds, the experimental evidence obtained from this study would seems to suggest that the compounds possess both centrally- and peripherally mediated anti-inflammatory effects. Therefore, it can be inferred that the substances liberated during inflammation, like cyclo-oxygenases, opiods, histamine and serotonin metabolite, are inhibited to produce the anti-inflammatory activity observed. This hypothesis is in consonance with observation of some previous workers (Eddy and Leimbark, 1953; Williamson. *et. al*. 1996).

In order to establish some structure-activity relationship (SAR) from the result presented in the study, the activity of the compounds are correlated with the functional groups at C-2 and C-4 position of the various aniline derivatives. The presence of chloro-group at C-4 position on the aniline in compound **13** could be responsible for its exceptional activity against the three models investigated for the anti-inflammatory activity. It has been reported from Quantitative structural activity relationship studies (QSARS) that the presence of an electron releasing group (by mesomeric effect) at position C-4 is identified for good anti-inflammatory activity (Hadjipavlou-Litina, 2000). It should be noted that the lesser activity for compounds **4** and **5** could be as a result of electron withdrawing effects of the $-COCH_3$ and the azo -group at position C-4. Comparing the structure of compound **13** with the standard drug indomethacin, there is a chloro group at C-4 position of the benzoyl group of indomethacin, which is similar to compound **13** (Figure 3).

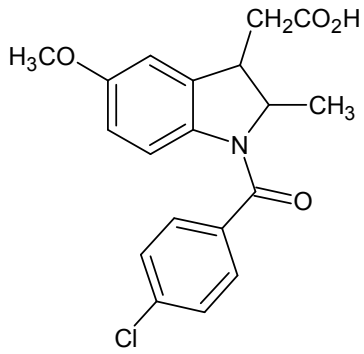


Figure 3: ***Indomethacin.***

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Declaration of Interest

The authors report no conflict of interest and are solely responsible for the contents and writing f the research article.

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