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Synthesis and Biological activities of some new Phthalides

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Abstract

A series of novel phthalide derivatives were prepared in one step, in moderate and good yields. The in vitro antibacterial and antifungal activities of these products were screened against two fungal strains (Thevialopsis paradoxa and Phomopsis mangiferae) and against bacterial strains (Staphylococcus aureus and Escherichia coli). The synthesized compounds were also tested for their analgesic and antioxidant activities. Structure activity relationship (SAR) reflects the effect of substituted phthalides on biological activity.

Keywords: o-aroyl benzoic acid, phthalide, antibacterial, antifungal, analgesic, antioxidant activities.

Introduction

Phthalides are a group of secondary metabolites or phytochemical compounds classified under lactones[1]. Phthalides are known to provide health benefits by stimulating and/or inhibiting various enzymes in the body[2]. Studies have shown that these compounds can help lower blood pressure, provide an anti-inflammatory function, improve circulation, rid the body of toxins such as uric acid crystals, inhibit malignancy and offer a calming effect[3-4].

Many of the major phthalides have been isolated from plants mainly medicinal herbs, celery stalks, celery seeds and essential oils of their plant of origin[5]. There are numerous reports on the bioactivities of phthalides but investigations concerning the mode of action are few.

Phthalides (isobenzofuranone), a family of five-membered lactones in plants, are important building blocks in a large number of biologically active compounds[6]. 3-Arylphthalides, for example, are useful intermediates for the synthesis of tri and tetracyclic natural products such as anthracycline antibiotics[7]. Phthalides are versatile starting materials for the synthesis of a variety of structures and the derivatives are also key intermediates for the synthesis of natural products[8]. Some 3-alkyl and 3-aryl substituted phthalides exhibit pharmacological applications, and some others have been used as starting materials for the synthesis of carbo- and heterocycles.

Prompted by the varied biological and herbicidal properties, the title compounds have been conveniently prepared by refluxing o-aroyl benzoic acid with substituted / unsubstituted bromo derivatives of acetophenone, propiophenone, coumarinyl acetophenone or diethyl bromomalonate in ethyl methyl ketone in the presence of K_2CO_3. Mixtures of two compounds phthalides, phenyl glyoxals (scheme Ia and Ib) / phthalide, propandione (scheme Ic) and phthalide, meso oxalic acid ester (scheme Id) was obtained, which were characterized by elemental and spectral analysis. Most of the phthalides were screened for their antimicrobial, analgesic and antioxidant activities but not the co prodcuts.

Material and Methods

General: The reagents and the solvents used in this study were of analytical grade and were used without further purification. Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was checked by TLC on silica gel GF254. IR were recorded on FTIR Perkin Elmer spectrophotometer. All the compounds gave satisfactory elemental analysis. Bromo derivatives **2**, **5**, **7** and **9** were prepared by literature method[9].

General procedure for the preparation of compounds 3-10: A mixture of phenanthrenoyl benzoic acid 1a (1g, 0.0033 moles) and Bromo derivatives 2 / 5 / 7 / 9 (0.919 g 0.0033 moles), anhy. K_2CO_3 (0.959g, 0.0069 moles) in ethyl methyl ketone was refluxed for 12- 15 hrs, solvent was then removed, water added and it was extracted with ethyl acetate. Solvent layer was first washed with saturated sodium bicarbonate and then with water and finally dried over anhy. Na_2SO_4 resulting in a mixture containing two compounds 3a and 4 / 6 / 8 / 10 (TLC). The two compounds were separated by column chromatography using petroleum ether (60-80^{0}C) -ethyl acetate.

3-Phenanthrenoyl phthalide 3a and 3g: white powder, mp 88^{0}C; 23.00%; Anal. Calcd $C_{22}H_{14}O_2$ (310.0 g): C, 85.16; H, 4.51; Found: C, 85.00; H, 4.57; IR (cm^{-1}): 1751, 1671 (five member lactone ring), 1532 (C=C); ^{1}H NMR δ 6.6 (s, 1H, C_3-H), 7.3-8.7 (m, 12H, aromatic protons), 8.0 (d, 1H, C_7-H); m/z: (M^+ - 1): 309.

3-Anthracenyl phthalide 3b and 3h: white powder, mp 72^{0}C; 37.58%; Anal. Calcd $C_{22}H_{14}O_2$ (310.0 g): C, 85.16; H, 4.51;

Found: C, 84.83; H, 4.73; IR (cm^{-1}): 1749, 1670 (five member lactone ring), 1487 (C=C); ^{1}H NMR δ 6.9 (s, 1H, C_3-H), 7.4-7.7 (m, 12H, aromatic protons), 8.20 (d, 1H, C_7-H); m/z: (M^+ + 2): 312.

3- (3' – hydroxy phenyl) phthalide 3c: pale yellow powder, mp 135^{0}C; 40.32%; Anal. Calcd $C_{14}H_{10}O_3$ (226.0 g): C, 74.34; H, 4.43; Found: C, 74.23; H, 4.40; IR (cm^{-1}): 1755, 1668 (five member lactone ring), 1501 (C=C), 3253 (OH); ^{1}H NMR δ 5.4 (s, 1H, OH), 6.65 (s, 1H, C_3-H), 6.6-7.4(m, 7H, aromatic protons), 8.3(d, 1H, C_7-H); m/z: (M^+): 226.

3-(2' – methyl – 5' – nitro benzoyl) phthalide 3d: yellow powder, mp 112^{0}C; 22.36%; Anal. Calcd $C_{15}H_{11}NO_3$ (269.0 g): C, 66.91; H, 4.08; N, 5.20; Found: C, 66.45; H, 4.20; N, 5.54; IR (cm^{-1}): 1755, 1676 (five member lactone ring), 1577 (C=C), 1380, 1583 (NO_2) ; ^{1}H NMR δ 2.2 (s, 3H, CH_3), 6.4 (s, 1H, C_3-H), 7.3-8.0 (m, 6H, aromatic protons), 8.1(d, 1H, C_7-H); m/z: (M^+ + 1): 270.

3-(2' – bromo – 4' – nitro benzoyl) phthalide 3e: yellow powder, mp 100^{0}C; 25.98%; Anal. Calcd $C_{14}H_8NBrO_3$ (334.0 g): C, 50.29; H, 2.39; N, 4.19; Found: C, 50.05; H, 2.50; N, 4.28; (cm^{-1}): 1743, 1644 (five member lactone ring), 1561 (C=C), 1300, 1605 (NO_2), 680 (C-Br) ; ^{1}H NMR δ 6.5 (s, 1H, C_3-H), 7.3-8.3 (m, 6H, aromatic protons), 8.0 (d, 1H, C_7-H); m/z: (M^+ - 1): 333.

3-Acetanilidyl phthalide 3f: yellow powder, mp 160^{0}C; 46.50%; Anal. Calcd $C_{16}H_{13}O_3N$ (267.0 g): C, 71.91; H, 4.86; N, 5.24; Found: C, 71.64; H, 5.24; N, 5.61; (cm^{-1}): 1705, 1689 (five member lactone ring), 1476 (C=C), 3361 (NH) ; ^{1}H NMR δ 3.40 (s, 3H, $COCH_3$), 6.60 (s, 1H, C_3-H), 7.60-8.20 (m, 7H, aromatic protons), 8.40-8.45 (dd, 1H, C_7-H), 13.90 (s, 1H, NH); m/z : (M^+ + 1): 268.

3-Dibenzofuryl phthalide 3i: white crystals, mp 108^{0}C; 68.00%; Anal. Calcd $C_{20}H_{12}O_3$ (300.0 g): C, 80.00; H, 4.00; Found: C, 80.36; H, 4.41; (cm^{-1}): 1774, 1636 (five member lactone ring), 1509 (C=C), 1259 (CO) ; ^{1}H NMR δ 6.5 (s, 1H, C_3-H), 7.3-8.0 (m, 10H, aromatic protons), 8.1 (d, 1H, C_7-H); m/z: (M^+ + 1): 301.

Results and Discussion

Chemistry: In experimental devised towards the synthesis of phthalides having aryl group at 3rd position of lactone ring. We had two synthetic methods in view: in first, refluxing o-aroyl benzoic acid with bromo derivative of different acetophenone / propiophenone / coumarinoyl acetophenone in ethyl methyl ketone in presence of base i.e anhydrous K_2CO_3, which always resulted in a mixture of two compounds (TLC), phthalide and phenyl glyoxal (scheme Ia), phthalide and propandione (scheme Ib), phthalide and coumarinoyl glyoxal (scheme Ic), and in second method refluxing o-aroyl benzoic acid with bromo diethyl malonate having same reaction medium as in first three resulted same phthalides but co- product formed here was meso oxalic acid ester (10) instead of phenyl glyoxal/ substituted phenyl glyoxal (4,6,8).

The formation of phthalide was by chance not by choice. In our previous work we have used o-acyl benzoic acid in place of o-aroyl benzoic acid which on refluxing with bromo derivatives of different acetophenone in same reaction condition resulted in formation of one single compound, isocoumarin[10-12], while on using o-aroyl benzoic acid, ring contraction took place and instead of single compound isocoumarin, mixture of two compounds was formed, which were judiciously separated using chromatography. The products formed were supported by spectral analysis. In IR, broad peak at 1736-1738 cm^{-1} suggested presence of five member lactone ring. The result apart from elemental analysis was supported by Mass spectrums where no peak beyond molecular weight of phthalide was found. The physical data was also in complete agreement with the products. In our earlier work we had already synthesized phthalides[13], in this paper some new phthalides have been introduced using different bromo derivatives as lactone ring system undoubtedly belongs to the most important heterocycles in nature, since it represents the main structure of many biologically significant compounds and are developed as pharmacologically active compounds or drugs. In light of above we synthesized some novel aroyl acids having nitrogen in the form of different functional groups such as $–NO_2$, $-NHCOCH_3$ to see its effect on biological terms. We have tried to see the biological effect in three ways, firstly by increasing fused benzene rings in phthalides for which we have used phenanthrene, anthracene, dibenzofuran, acetanilide, nitro / aroyl acid, secondly by introducing nitrogen containing functional groups in phthalides and lastly using coumarin moiety in form of its bromo derivative which can show biological effect in terms of increased benzene ring and also in terms of additional oxygen heteroatom.

The formation of phthalides from o-aroyl benzoic acid is also supported[14], as o-aroyl benzoic acid exists as phthalide in its tautomeric form. Apart from this steric / electronic factor might be working in phthalide formation.

Biological Assays: Antibacterial activity of the target compounds were tested in vitro against bacterial strains *E. Coli* (gram negative) and *S. Aureus* (gram positive) using serial agar dilution (cup plate method)[15].

The two microorganisms were cultured in dishes containing agar medium. Four cups (8 mm) were put onto the dishes and each tested compound (0.1ml of 2mg/ml) was then added into the cups under aseptic condition. Then the dishes were incubated at 37^{0}C for 24h. The zone of inhibition of the growth of the bacteria, which was produced by diffusion of the compounds from the cup into the surrounding medium, was measured in milli meters to evaluate the antibacterial activity. Each experiment was repeated twice. DMSO was used as a positive control for all the experiments.

The standard fungal culture *T. Paradoxa and P. Mangiferae* were grown on PDA slants at room temperature. Mycelial growth inhibition of *T. Paradoxa and P.Mangiferae* was evaluated by the poisoned food technique[16], where the inhibition in growth of the fungal strain was observed on PDA. The stock solutions (1000ppm) were made from each of the test compounds. The required % concentrations of the compounds (mg/ml) were obtained by mixing the appropriate amount of the stock solution with 20 ml of molten PDA. The amended PDA was poured into Petri dishes and allowed to set.

An inoculum of the fungus obtained from 7 days old axenic culture, grown as above, was placed at the centre of the amended agar medium. Each experiment was performed in triplicate. The diameter of the fungal colony was measured after 4 days, then after 7 days at $26+1^0$C and the % inhibition was calculated using the following equation:

$$\% \text{ inhibition } = \frac{\text{Growth area in reference } - \text{ growth area in sample}}{\text{Growth area in reference}} \times 100$$

Analgesic activity of the compounds was determined by Tail flick method[17]. Mice of either sex weighing between 20-25 g which shows positive response were selected and divided into different groups with four mice in each group. The first group served as control which received 2% gum acacia. Second group served as standard which received analgin at a dose of 50 mg/kg body weight orally. Rest of the groups received test compounds at a dose of 50 mg/kg body weight of mouse, orally.

The tail of the mouse was dipped (up to 5 cm) in a water bath at 55 ± 0.7^0C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cut-off time being 60 seconds. The first reading was taken immediately after administration of the standard drug and test compounds and afterwards at the intervals of 30 minutes. The response time was recorded and the results are described in tabular form.

Antioxidant activity was tested by estimating scavenging activity for nitrous oxide using Griess reagent[18] by test compounds.

2.0 ml of sodium nitroprusside (10 mM) in 0.5ml phosphate buffer pH 7.4 was incubated with 0.5 ml, 1000 ppm concentration of test compounds dissolved in a suitable solvent (DMSO) and tubes were incubated at 25^0C for 150 min. Control experiment was conducted with equal amount of solvent in an identical manner. After 150 mins, 1.0 ml of incubation solution was taken and diluted with 4.0 ml of Griess reagent (1.0 ml 1% sulfanilamide, 1.0 ml 5% o-phosphoric acid and 2.0 ml 0.1% N-naphthylethylenediamine dihydrochloride dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent N-naphthylethylenediamine dihydrochloride was read at λ 546 nm after 30 mins. The experiment was repeated in triplicate. % NO scavenging was calculated using the following equation:

$$\% \text{ No Scavenging } = \frac{\text{Acontrol} - \text{A test}}{\text{Acontrol}} \times 100$$

The most tested compounds show growth inhibitory action for fungus *P. Mangiferae*. However, they have no effects on bacteria *S. Aureus* except 3a. But against gram negative bacteria *E. Coli,* except 3a all compounds showed good zone of inhibition. In analgesic activity, increase in number of fused benzene rings helps in increasing the analgesic effect (3d, 3i) and compounds where apart from oxygen, nitrogen hetro atom or –Br were present (3d, 3f, 3i). Antioxidant activity was excellent with 3i where electronegative –Br atom was present as substituted group supporting electron density which take part in most of the biological activity and moderate to good with other compounds with respect to control.

Table-1
Antimicrobial Activity

Compound	Zone of Inhibition (mm)		% Growth inhibition	
	S. Aureus	***E. Coli***	***T. Paradoxa***	***P. Mangiferae***
3a	16	-	51.09	57.00
3b	13	10	21.99	40.03
3d	12	13	11.04	11.25
3f	10	13	40.21	42.65
3i	11	13	24.93	36.04
3j	12	13	41.48	46.00
Control	-	-	-	-
Ampicillin	15	5	-	-

Conclusion

In conclusion, new phthalide derivatives were prepared from easily accessible starting materials in single step. The preliminary *in vitro* test results of these compounds against the four studied micro-organisms such as *Staphylococcus aureus and Escherichia coli* and *Thevialopsis paradoxa and Phomopsis mangiferae* shows significant activity. All the tested compounds showed good analgesic and antioxidant action.

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Table-2
Analgesic activity

Compound	Dose (mg/kg) body weight	Average (± SE) reaction time (sec.) Time after drug treatment (min.)			
		0	**30**	**60**	**90**
Control	50	3.01 (± 0.358)	3.20 (± 0.288)	3.10 (± 0.358)	3.02 (± 0.00)
Standard	50	3.09 (± 0.408)	5.25 (± 0.249)	7.75 (± 0.249)	9.00 (± 0.000)
3a	50	3.27 (± 0.277)	3.98 (± 0.153)	6.74 (± .540)	8.14 (± 0.560)
3b	50	3.03 (± 0.008)	5.49 (± 0.049)	7.02 (± 0.195)	8.63 (± 0.403)
3d	50	3.00 (± 0.408)	3.32 (± 0.408)	3.59 (± 0.549)	3.31 (± 0.408)
3f	50	2.51 (± 0.408)	4.06 (± 0.408)	5.77 (± 0.749)	7.10 (± 0.408)
3i	50	3.51 (± 0.418)	4.00 (± 0.200)	5.59 (± 0.142)	6.34 (± 0.208)
3j	50	2.31 (± 0.249)	4.52 (± 0.408)	6.52 (± 0.408)	6.26 (± 0.353)

Table-3
Antioxidant activity

Compound	Absorbance	% NO Scavenging
3b	0.1806	84.45
3e	0.4798	58.69
3i	-0.1110	109.55
3j	0.3052	73.72
Control	1.1615	-

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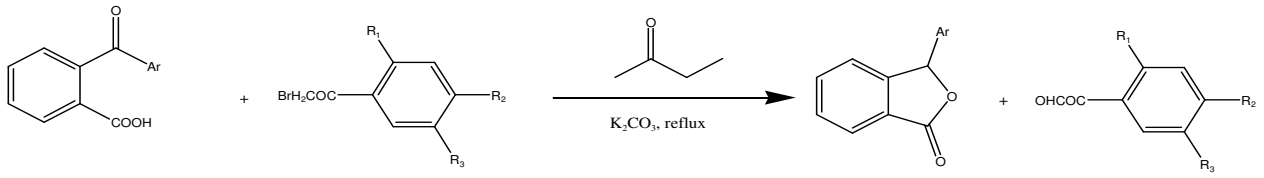
Scheme Ia

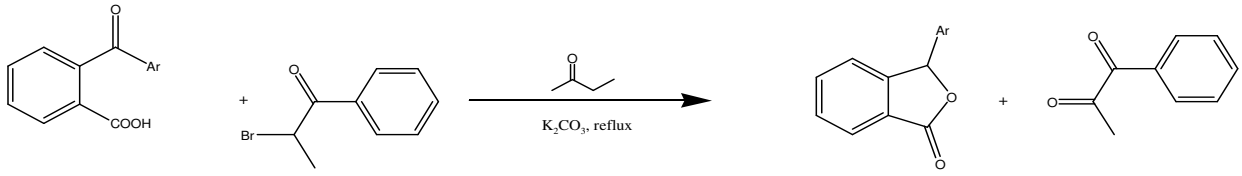


1 2 3 4

1a & 3a, Ar = phenanthrene
1b & 3b, Ar = anthracene
1c & 3c, Ar = m-hydroxy benzene
1d & 3d, Ar = p-nitro toluene
1e & 3e, Ar = m-bromo nitrobenzene
1f & 3f, Ar = acetanilide
1g & 3g, Ar = phenanthrene
1h & 3h, Ar = anthracene
1i & 3i, Ar = bromobenzene

2a & 4a, $R_1 = R_2 = R_3 = H$
2b & 4b, $R_1 = R_2 = R_3 = H$
2c & 4c, R_1 =OH, $R_2 = H$, $R_3 = CH_3$
2d & 4d, $R_1 = R_3$ =H, R_2= OH
2e & 4e, $R_1 = R_3$ =H, R_2= OH
2f & 4f, $R_1 = R_3$ =H, R_2= OH
2g & 4g, $R_1 = R_3$ =H, R_2= OH
2h & 4h, $R_1 = R_3$ =H, R_2= OH
2i & 4i, $R_1 = R_2 = R_3 = H$

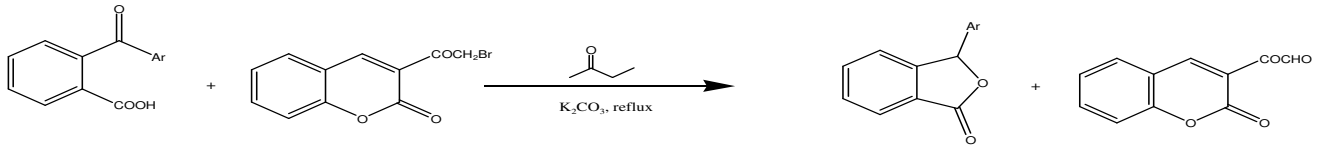
Scheme Ib



1 5 3 6

1a & 3a, Ar = phenanthrene
1b & 3b, Ar = anthracene
1c & 3c, Ar = m-hydroxy benzene
1d & 3d, Ar = p-nitro toluene
1e & 3e, Ar = m-bromo nitrobenzene
1j & 3j, Ar = dibenzofuran

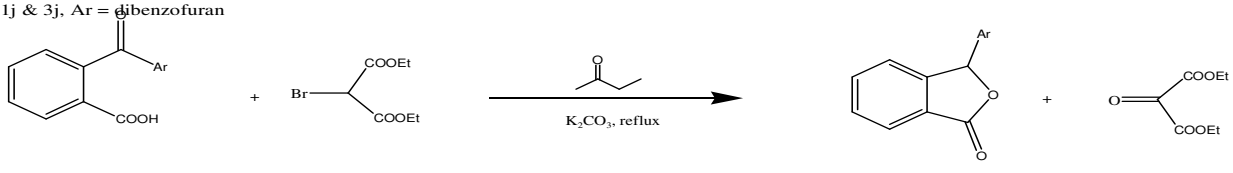
Scheme 1c



1 7 3 8

1a & 3a, Ar = phenanthrene
1b & 3b, Ar = anthracene
1c & 3c, Ar = m-hydroxy benzene
1d & 3d, Ar = p-nitro toluene
1e & 3e, Ar = m-bromo nitrobenzene
1j & 3j, Ar = dibenzofuran

Scheme Id



1 9 3 10

1a & 3a, Ar = phenanthrene
1b & 3b, Ar = anthracene
1c & 3c, Ar = m-hydroxy benzene
1d & 3d, Ar = p-nitro toluene
1e & 3e, Ar = m-bromo nitrobenzene
1j & 3j, Ar = dibenzofuran