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ORIGINAL RESEARCH

A facile synthesis of new 3,3-disubstituted phthalides of pharmacological interest

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Abstract A series of new phthalides of pharmacological interest were synthesized by a protocol involving condensation of two γ-keto acids, 2-(4-isopropylbenzoyl)benzoic acid (**1a**) and 2-(4-isopropyl-3-nitrobenzoyl)benzoic acid (**1b**) with phenolic compounds in the presence of catalytic quantity of concentrated sulphuric acid. The method is simple, efficient, economical and environmentally benign as the reaction is carried out under solvent free condition attempting a green approach. Structural characterization of these newly synthesized compounds was accomplished by IR, UV, ^{1}H NMR, ^{13}C NMR, Mass spectral data, elemental analysis and chemical reactions. Some of the synthesized phthalides were found to exhibit antifungal and antibacterial activity against various human pathogenic bacterial and fungal strains.

Keywords Phthalides · Phenolic compounds · 2-(4-Isopropylbenzoyl)benzoic acid · 2-(4-Isopropyl-3-nitrobenzoyl)benzoic acid · Antibacterial and antifungal activities

Introduction

Phthalides constitute an important group of biologically active compounds which are associated with a wide range of pharmacological activities including action on central nervous system, anti-angina, anti-platelet aggregation, anti-smooth muscle proliferation, anti-thrombosis, cardiac function modulation and protection against cerebral ischemia (Lin *et al.*, 2005). The rhizome of *Ligusticum wallichii* (family-Umbelliferae) has been used by Chinese for several thousand years to relieve headache, abdominal pain, cardiovascular and gynaecological diseases (Ko *et al.*, 1983). The butylidenephthalide, the most potent antispasmodic agent isolated from this crude drug was prepared by a synthetic method and found to be useful as a coronary dilator and antihypertensive drug (Ko *et al.*, 1994). Chiral 3-substituted phthalides are found to possess significant pharmacological properties and are also used as versatile building blocks for large number of medicinally important compounds (Knepper *et al.*, 2004; Witulski *et al.*, 2002). Natural products like 3-butylphthalide have been successfully synthesized due to their potent biological activities (Zhang *et al.*, 2010). Flexible racemic synthesis of phthalide-containing antibiotics that inhibit *Helicobacter pylori* have been carried out in a convergent fashion by Wittig coupling (Brimble *et al.*, 2005). Antimicrobial activity of celery (*Apium graveolens*) leaves and roots is also due to the presence of 3-substituted phthalides in them (Sipailiene *et al.*, 2005).

During recent years, there has been a remarkable attraction towards the development of convenient and efficient methodologies for the synthesis of phthalides (Zhang *et al.*, 2009; Karnik and Kamath, 2008; Lin and Sun, 2008; Zhou and Jiang, 2007; Patil and Karnik, 2007; Chang *et al.*, 2007; Pedrosa *et al.*, 2006; Huang *et al.*, 2006; Kosoka *et al.*, 2005; Tanaka *et al.*, 2004; Witulski and Zimmerman, 2002; Lei *et al.*, 2002; Everaere *et al.*, 2001). The above mentioned literature reports related with the wide spectrum biological activities of phthalides encouraged us to undertake the synthesis of some new 3-substituted phthalides which are expected to have a number of pharmacological applications. In these compounds carbon-3 of the phthalide structure is attached with

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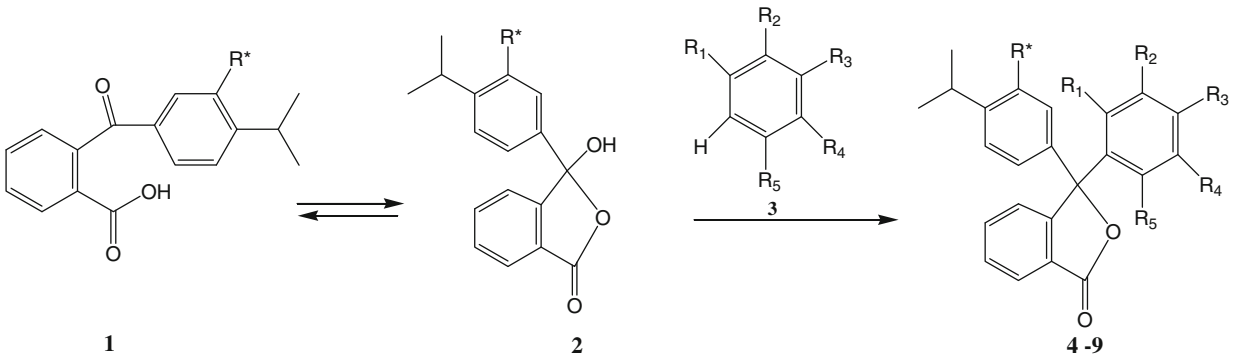
cumene and phenolic structural moieties. Cumene containing essential oils have been found to exhibit antibacterial activity, and hence, it is used in bioremediation studies. Bacteria used in the bioremediation of trichloroethylene exhibited an increased capability to degrade trichloroethylene in the presence of cumene (Suttinun *et al*., 2009).

Phenols and their derivatives form an important group of compounds possessing biological activities. Various plant flavonoids are reported to have antitumour, antioxidant and antimicrobial activities due to the presence of a phenolic moiety in their structure (Seeram *et al*., 2006). Pathogenesis of many chronic diseases, such as cancer, cardiovascular and neurodegenerative diseases caused by reactive oxidative species has attracted much attention and require novel antioxidants to combat the reactive oxidative species (Shahidi, 1997; Thomas, 1997). Some antioxidant pharmacophores have been identified to facilitate the discovery of antioxidants, among which catechol has been given the highest attention (Zhang, 2005, 2000; Zhang et al., 2003a, b; Foti *et al*., 2002; Hussain *et al*., 2003; Chichirau *et al*., 2005; Flueraru *et al*., 2005). Catechol and pyrogallol are reported as allelochemicals belonging to the group of phenolic compounds synthesized in plants possessing antimicrobial activities (Kocaçalışkan *et al*., 2006). Resorcinol and phenol derivatives from plant sources have been found to have anticancer and antibacterial activities (Barbini *et al*., 2006; Pormord *et al*., 2006; Mekawey *et al*., 2009). Both synthetic and natural phloroglucinol compounds exhibit biological properties such as antispasmodic, antiviral, antiprotozoal and antimicrobial activities (Singh *et al*., 2009).

From the foregoing account, it is amply evident that phthalides, phenolic compounds and cumene or isopropylbenzene are bestowed with excellent medicinal properties. The molecular manipulation of promising lead compounds continues to be a widely used approach for the development of new or better drugs. This approach aims at combination of two or more different pharmacophoric units or groups into a single entity having more potent or entirely new therapeutic properties. Thus, it is anticipated that addition of hydroxyphenyl and isopropylbenzene moieties to the phthalide structure, will enhance the biological activity of these compounds. The synthesized compounds may be regarded as 'phenol–phthalide–cumene conjugates'. A few of the synthesized compounds were evaluated for their antibacterial and antifungal activities.

Chemistry

The synthesis of 3,3-disubstituted phthalides (**4a–9a** and **4b–9b**) was accomplished by condensing two γ-keto acids, 2-(4-isopropylbenzoyl)benzoic acid (**1a**) and 2-(4-isopropyl-3-nitrobenzoyl)benzoic acid (**1b**), with various phenols (phenol, resorcinol, catechol, quinol, phloroglucinol and pyrogallol) in the presence of catalytic quantity of concentrated sulphuric acid under solvent free reaction condition attempting to follow a green approach. The γ-keto acids **1a** and **1b** reacted with phenols (**3a–f**) through their lactol tautomeric form **2a** and **2b**, respectively, to give the desired phthalides (**4a–9a** and **4b–9b**) as depicted in Scheme 1. Their physical and analytical data are presented in Table 1. The occurrence of keto-lactol tautomerism in γ-keto acids and γ-formyl acids is thoroughly investigated, and it is well known that in many chemical reactions these acids participated through their cyclic lactol form (Valter and Flitsch, 1985; Zhang et al., 2003a, b). The phenols were taken in slight excess of molecular proportion than the γ-keto acids, and with excess phenols, the whole acid



3a, 4 : R_1=R_2=R_4=R_5=H; R_3=OH
3b, 5 : R_2=R_4=R_5=H; R_1=R_3=OH
3c, 6 : R_1=R_4=R_5=H; R_2=R_3=OH

3d, 7 : R_2=R_3=R_5=H; R_1=R_4=OH
3e, 8 : R_2=R_4=H; R_1=R_3=R_5=OH
3f, 9 : R_4=R_5=H; R_1=R_2=R_3=OH

1a, 2a, **4a-9a** (R* = H) and **1b, 2b, 4b-9b** (R* = NO_2)

Scheme 1 Synthesis of phthalides **4–9**

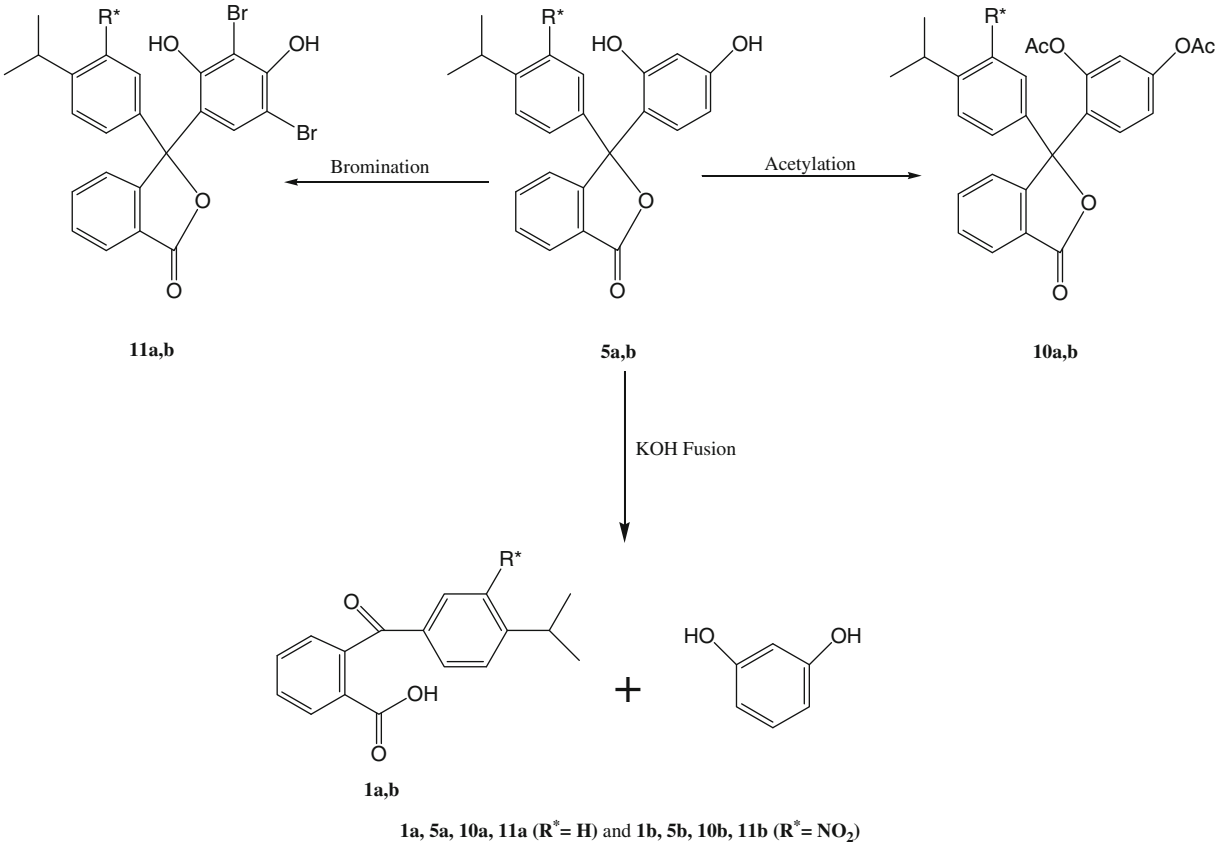
Table 1 Physical and analytical data of phthalides **4a–9a** and **4b–9b**

	Phthalide	Condensation		Appearance (microcrystalline)	m.p. (°C)	Yield (%)	Mol. Formula	Analysis (%)		
		Temp. (°C)	Duration (h)					Found (Calcd.)		
								C	H	N
4a	3-(*p*-Hydroxyphenyl)-3-(4-isopropylphenyl)phthalide	130–140	5	Light brown	140–142	89	$C_{23}H_{20}O_3$	80.41 (80.21)	5.78 (5.85)	–
4b	3-(*p*-Hydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide	110–120	3	Pinkish brown	200–202	56	$C_{23}H_{19}NO_5$	70.99 (70.94)	4.96 (4.92)	3.68 (3.60)
5a	3-(2,4-Dihydroxyphenyl)-3-(4-isopropylphenyl)phthalide	110–120	0.5	Pinkish brown	162–164	98	$C_{23}H_{20}O_4$	76.41 (76.65)	5.47 (5.59)	–
5b	3-(2,4-Dihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide	125–135	0.5	Purple	180–182	74	$C_{23}H_{19}NO_6$	68.21 (68.14)	4.79 (4.72)	3.57 (3.46)
6a	3-(3,4-Dihydroxyphenyl)-3-(4-isopropylphenyl)phthalide	105–115	1.5	Grey	135–137	76	$C_{23}H_{20}O_4$	76.50 (76.65)	5.38 (5.59)	–
6b	3-(3,4-Dihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide	120–130	1.5	Dark brown	280–282	50	$C_{23}H_{19}NO_6$	68.22 (68.14)	4.81 (4.72)	3.55 (3.46)
7a	3-(2,5-Dihydroxyphenyl)-3-(4-isopropylphenyl)phthalide	140–150	1.5	Light brown	200–202	65	$C_{23}H_{20}O_4$	76.74 (76.65)	5.61 (5.59)	–
7b	3-(2,5-Dihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide	130–140	1.5	Brown	170–172	50	$C_{23}H_{19}NO_6$	68.20 (68.14)	4.79 (4.72)	3.57 (3.46)
8a	3-(2,4,6-Trihydroxyphenyl)-3-(4-isopropylphenyl)phthalide	170–180	1	Brownish orange	230–232	98	$C_{23}H_{20}O_5$	73.27 (73.39)	5.28 (5.36)	–
8b	3-(2,4,6-Trihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide	180–190	0.5	Brown	>300	64	$C_{23}H_{19}NO_7$	65.70 (65.55)	4.60 (4.54)	3.46 (3.32)
9a	3-(2,3,4-Trihydroxyphenyl)-3-(4-isopropylphenyl)phthalide	135–145	0.5	Brownish green	185–187	86	$C_{23}H_{20}O_5$	73.60 (73.39)	5.36 (5.36)	–
9b	3-(2,3,4-Trihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide	120–130	0.5	Green	>300	56	$C_{23}H_{19}NO_7$	65.65 (65.55)	4.65 (4.54)	3.49 (3.32)

taken reacted as lactol. The homogeneity of the synthesized products was ascertained by TLC. Their structures were elucidated by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR, Mass spectral data and chemical reactions such as acetylation, bromination and caustic potash fusion. The representative phthalides **5a** and **5b** when subjected to acetylation and bromination afforded their corresponding diacetyl (**10a** and **10b**) and dibromo (**11a** and **11b**) derivatives, respectively, while on fusion with caustic potash each of them was degraded into the starting γ-keto acid **1a** or **1b** and resorcinol as shown in Scheme 2.

Results and discussion

The synthesis of phthalides (**4a–9a** and **4b–9b**) involves a condensation reaction in which γ-keto acids, 2-(4-isopropylbenzoyl)benzoic acid (**1a**) and 2-(4-isopropyl-3-nitrobenzoyl)benzoic acid (**1b**) reacted with phenols (**3a–f**) through their cyclic lactol tautomeric forms **2a** and **2b**, respectively, as shown in Scheme 1. The phthalide **5a, b** on acetylation and bromination afforded diacetyl (**10a, b**) and dibromo (**11a, b**) derivatives, respectively, as given in Scheme 2. All the synthesized compounds (**4–11**) are the unsymmetrically substituted phthalides in which carbon-3 of phthalide structure is attached to two different phenyl nuclei, one being phenolic bearing one, two or three hydroxyl groups, and the other non-phenolic having a isopropyl group with or without nitro group. During the present study, in some of the synthesized phthalides, a nitro group has also been incorporated in the cumene moiety because in many molecules nitro group has been found to act as a pharmacophore. Chloramphenicol is a fascinating example of a naturally occurring medicinally useful nitro compounds. The antiproliferative effect of some phthalide derivatives possessing nitro group have shown cytotoxicity



Scheme 2 Acetylation, bromination and KOH fusion of phthalides **5a**, **b**

against human tumour cell lines (Kohn *et al*., 2006). High physiological activity in the compounds containing nitro group (nitrophenols, nitrobenzenes and nitrofurans) is well known.

Taking into account the various useful pharmacological properties reported for phenols, cumene and phthalides, the synthesized compounds are expected to be potentially useful therapeutic agents. In order to detect the possibility of antimicrobial activity in these compounds, a few of them were evaluated for in vitro antimicrobial activity on the basis of their IC_{50} and MIC values which were determined by standard procedures. Results of their antibacterial and antifungal activities are presented in Tables 2 and 3.

The physical and analytical data of the synthesized phthalides are given in Table 1. The structures of all the phthalides were established by their analytical and spectral data (IR, UV, ^{1}H NMR, ^{13}C NMR, Mass). IR spectra of the synthesized phthalides **4a**–**9a**, **4b**–**9b** and **11a, b** exhibited a strong and broad band in the region between 3,250 and 3,422 cm^{-1} due to OH stretching vibrations. The broad

Table 2 Antifungal and antibacterial activity of some of the synthesized phthalides (IC_{50} μg mL^{-1})

Compounds	IC_{50} (μg mL^{-1})									
	C. albicans	*C. glabrata*	*C. krusei*	*A. fumigatus*	*C. neoformans*	*S. aureus*	MRS	*E. coli*	*P. aeruginosa*	*M. intracellulare*
6a	172.41	45.81	15.46	53.23	137.3	4.96	6.81	>200	60.97	>200
6b	>200	76.28	>200	>200	>200	26.36	27.79	>200	>200	>200
9a	65.02	17.5	25.48	>200	>200	18.45	18.8	169.8	116.4	>200
9b	98.94	21.32	88.67	>200	>200	42.3	45.7	>200	162.8	>200
Amphotericin B	0.20	0.28	0.56	0.67	0.32	–	–	–	–	–
Ciprofloxacin	–	–	–	–	–	0.11	0.10	0.005	0.11	0.32

Table 3 Antifungal and antibacterial activity of selected of the synthesized novel phthalides (MIC μg mL^{-1})

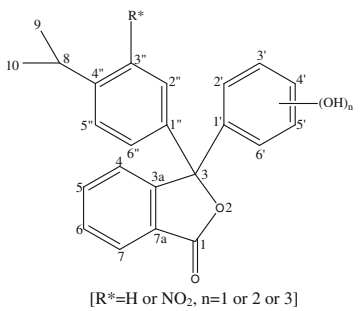
Compounds	MIC (μg mL^{-1})									
	C. albicans	*C. glabrata*	*C. krusei*	*A. fumigatus*	*C. neoformans*	*S. aureus*	MRS	*E. coli*	*P. aeruginosa*	*M. intracellulare*
6a	>200	200	>200	>200	>200	18.75	12.50	>200	>200	>200
6b	>100	>100	>100	>100	>100	50.00	50.00	>200	>200	>200
9a	>200	>200	>200	>200	>200	25.00	25.00	>200	>200	>200
9b	>200	83.33	>200	>200	>200	100	133.33	>200	>200	>200
Amphotericin B	0.52	0.63	1.25	1.25	0.63	–	–	–	–	–
Ciprofloxacin	–	–	–	–	–	0.33	0.42	0.013	0.83	0.50

envelope and low frequency of this band indicated that OH groups are either involved in strong hydrogen bonding or tautomeric shifts. This broad band between 3,250 and 3,422 cm^{-1} was found to be absent in **10a, b**, instead these compounds displayed a strong band at 1,755 or 1,760 cm^{-1} assignable to carbonyl stretching in a phenolic acetate moiety. All the phthalides, **4–11** showed a sharp and strong band near 1,740–1,760 cm^{-1} which could be attributed to the lactonic carbonyl group present in all the structures. The stretching vibrations of C–O–C of the lactonic structures gave a peak at 1,000–1,020 cm^{-1} and C–O bond stretching of the phenolic group gave absorption peaks near 1,108–1,120 and 1,250–1,260 cm^{-1}. The presence of two bands at 690–700 cm^{-1} and 750–760 cm^{-1} in all the compounds is in accordance with the o-disubstituted phthalide ring of their structures. The nitro group attached to phenyl group in the structures of **4b–11b** gave three IR absorptions near 1,530–1,550; 1,330–1,350 and 840-850 cm^{-1} that can be assigned to asymmetric N–O stretching, symmetric N–O stretching and C–N stretching vibrations, respectively. The UV spectra (in methanol) of all the compounds revealed the same pattern of absorption at 208–225, 245–290 and 285–340 nm. In the ^{1}H NMR spectra (400 MHz, DMSO-d_6) of the synthesized phthalides **4–11**, the aromatic protons displayed a complex multiplet in the region δ 6.20–8.15. The hydroxyl protons in **4a–9a**, **4b–9b** and **11a, b** appeared as a singlet from δ 5.70 to 9.50. The phthalides **10a, b** did not give any signal for hydroxyl protons, instead, these displayed a sharp singlet at δ 1.20 or 1.30 due to the presence of acetoxyl protons. ^{1}H NMR spectra of all the phthalides (**4–11**) showed a doublet at δ 1.10–1.30 and septet at δ 2.50–3.20 which are given by methyl protons and methine proton, respectively, present in the isopropyl group. ^{13}C NMR spectral data of all the compounds are also in good agreement with the structures assigned to them. For the purpose of assignment of ^{13}C NMR spectral data, the carbon atoms of the synthesized phthalides, **4–9** (Scheme 1) are numbered according to the pattern shown in Fig. 1. Spectral data and their assignments are given in experimental section.

The representative compounds **5a** and **5b** were subjected to mass spectral analysis. The compound **5a** gave molecular ion peak at *m/z* 359 $(M-H)^-$ together with a base peak at *m/z* 317 and other peaks at *m/z* 342, 299, 273 and 239. The compound **5b** gave a peak at *m/z* 404 $(M-H)^-$ and a base peak at *m/z* 91 with other peaks at *m/z* 297, 208, 117. Mass spectrum of the compound **5a** is shown in Fig. 2 while the proposed fragmentation pattern is depicted in Scheme 3.

Experimental

All the melting points were determined in open capillary tubes and are uncorrected. UV spectra were taken in methanol on a Perkin-Elmer Lambda 15 UV/Vis spectrophotometer. IR spectra were recorded in KBr on a Perkin-Elmer FT-IR Spectrum RX-I spectrometer. The ^{1}H NMR spectra were recorded in DMSO-d_6 solutions on a Bruker 400 NMR spectrometer using TMS as internal standard. ^{13}C NMR spectra were measured at 100 MHz in DMSO-d_6. Mass spectra were recorded on a GC–MS spectrometer (Agilent 6890N GC), equipped with 5973 inert mass selective detector, Agilent Technologies, USA. The samples were dissolved in dichloromethane and they were



[R*=H or NO_2, n=1 or 2 or 3]

Fig. 1 Numbering of carbon atoms for ^{13}C NMR spectral assignment

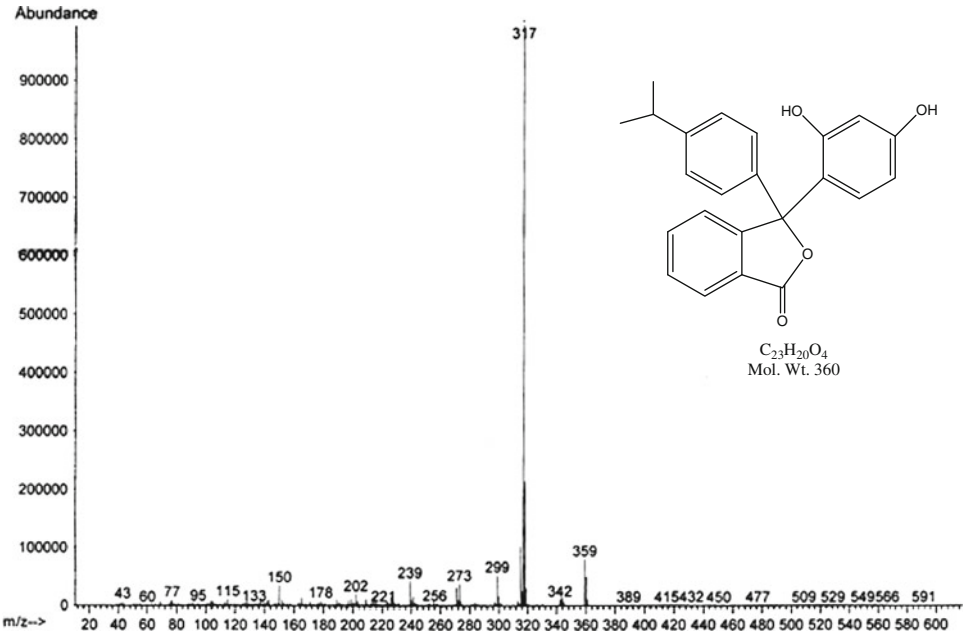


Fig. 2 Mass spectrum of **5a**

injected into GC–MS at an injection volume of 1 μL. The MS was operated at a voltage of 70 eV. Ionization was effected by electro spray ionization technique. The chemicals and solvents were procured from E. Merck (Germany) and Qualigens (India) and used as received without further purification. The progress of the reactions and homogeneity of the products was ascertained by TLC. For TLC, plates coated with alumina–silica gel G (1:1) layers were run in ethyl acetate–methanol–5 N ammonia (40:40:20).

The starting γ-keto acid, 2-(4-isopropylbenzoyl)benzoic acid (**1a**) was synthesized by Friedel–Crafts phthaloylation of cumene by modifying the reported procedure (Underwood and Walsh, 1935), to get better yield, and to avoid the formation of sticky and impure product. The acid **1a** was subjected to nitration to get 2-(4-isopropyl-3-nitrobenzoyl)benzoic acid (**1b**).

2-(4-Isopropylbenzoyl)benzoic acid (**1a**)

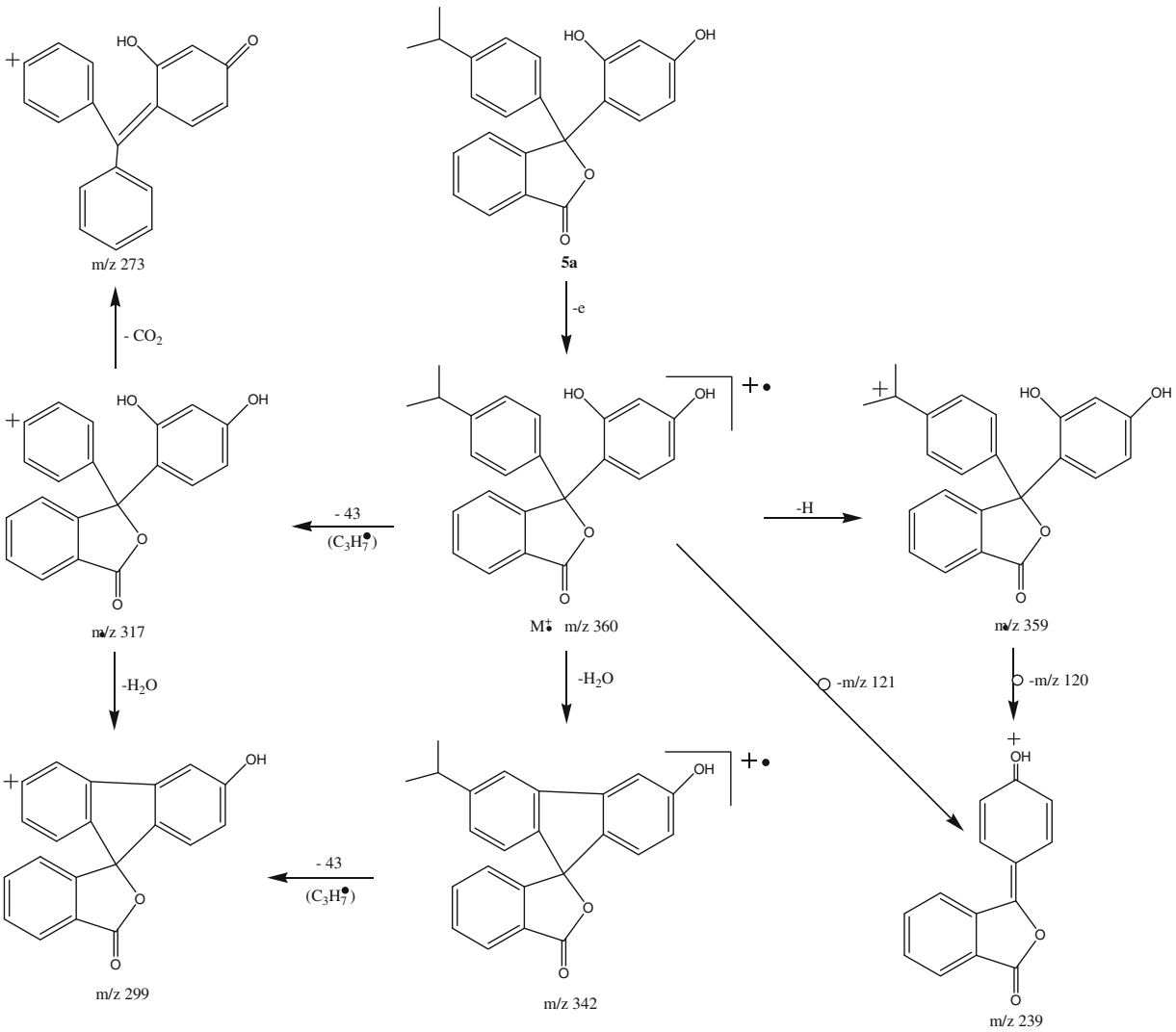
A mixture of finely powdered phthalic anhydride (22.2 g, 0.15 mol), cumene (18 g, 0.15 mol) and carbon disulphide (200 ml) was vigorously agitated for 1 h. Then anhydrous aluminium chloride (40.2 g, 0.3 mol) was added gradually with stirring and maintaining the reaction temperature below 40 °C. The mixture was then gently refluxed on a steam bath for 3 h and allowed to stand overnight. It was decomposed with ice and concentrated hydrochloric acid, and carbon disulphide was distilled off. The semi solid mass left behind was cooled in an ice-bath and filtered. The residue was thoroughly washed with cold water and extracted with 5 % (w/v) warm aqueous solution of anhydrous sodium carbonate. The extract was boiled with animal charcoal, filtered, cooled and acidified with concentrated hydrochloric acid. The precipitated **1a** was crystallized from the ethanol as yellowish white crystalline solid (35 g, 86 %), m.p. 136–137 °C [Ref. (Underwood and Walsh, 1935) m.p. 133–134 °C].

2-(4-Isopropyl-3-nitrobenzoyl)benzoic acid (**1b**)

2-(4-Isopropylbenzoyl)benzoic acid (26.8 g, 0. 1 mol) was dissolved in concentrated nitric acid (d, 1.41, 100 ml), the resulting solution was refluxed for 1 h and then cooled in an ice bath. The separated solid was filtered and washed with water and dried. Crystallization of the product from ethanol gave **1b** as yellowish-white crystals (18.23 g, 68.1 %); m.p. 160–162 °C. IR (KBr, ν, cm^{-1}): 3400, 2972, 2800, 2700, 1780 (weak), 1690, 1675, 1600, 1580, 1530, 1490, 1450, 1360, 850. Anal. calcd. for $C_{17}H_{15}NO_5$: C, 65.17; H, 4.83; N, 4.47. Found C, 65.90; H, 4.32; N, 4.65 %.

General procedure for the synthesis of phthalides (**4–9**)

An intimate mixture of γ- keto acid **1a** or **1b** (0.01 mol) and phenolic compound **3a–f** (0.015 mol) was heated in an oil bath to afford a homogeneous molten mass. To this, concentrated sulphuric acid (3–5 drops) was added cautiously with stirring by a glass rod, and the heating was continued for 0.5–5 h (monitored by TLC) to obtain a hard and brittle mass on cooling. The condensed mass so obtained was crushed and thoroughly washed with water to remove the excess phenolic compounds. In case of synthesis of **4a**, **b**, the unreacted phenol was removed by steam distillation. Then the product was extracted with 2 % aqueous NaOH and the extract was filtered. Acidification



Scheme 3 Possible mass fragmentation pattern for **5a**

of the filtrate by gradual addition of dil. HCl precipitated the phthalides (**4**–**9**). They were purified by repeated crystallization with aqueous ethanol, dried in an oven at 100 °C and finally over phosphorus pentoxide in a vacuum desiccator. Preparatory conditions and physical data of the synthesized phthalides are presented in Table 1. The results of their spectral studies are given below.

3-(*p*-Hydroxyphenyl)-3-(4-isopropylphenyl)phthalide (**4a**)

IR (KBr, ν, cm^{-1}): 3393 (OH), 2962 (aliphatic C–H), 1744 (lactonic >C=O), 1610, 1590, 1513, 1466 (Ar C=C), 1255, 1120 (C–O), 1018 (C–O–C), 760, 693 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 210, 265, 310 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.1 (d, 6H, 2× CH_3, J = 6.5 Hz), 2.9 (sept, 1H, –CH<), 6.7 (s, 1H, OH), 6.8–8.0 (m, 12H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.2 (C-1), 156.8 (C-4′), 155.5 (C-3a), 147.1 (C-4″), 140.2 (C-1″), 134.5 (C-5), 132.3 (C-1′), 131.7 (C-2″/C-6″), 130.1 (C-2′/C-6′), 129.9 (C-7), 128.2 (C-4), 126.9 (C-7a), 126.1 (C-6), 125.6 (C-3″/C-5″), 115.4 (C-3′/C-5′), 80.9 (C-3), 36.0 (C-8), 24.1 (C-9/C-10).

3-(*p*-Hydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide (**4b**)

IR (KBr, ν, cm^{-1}): 3409 (OH), 2920 (aliphatic C–H), 1750 (lactonic >C=O), 1600, 1590, 1500, 1450 (Ar C=C), 1550 (asym. N–O), 1350 (sym. N–O), 1255, 1108 (C–O), 1020 (C–O–C), 840 (C–N), 755, 690 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 218, 275, 325 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.2 (d, 6H, 2× CH_3, J = 6.5 Hz), 2.7 (sept, 1H, –CH<), 6.7–8.0 (m, 11H, Ar–H), 9.7 (s, 1H, OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169.1 (C-1), 158.5 (C-4′), 152.9 (C-3a), 146.9

(C-3″), 142.1 (C-1″), 141.5 (C-4″), 134.9 (C-5), 132.0 (C-1′), 130.5 (C-2′/C-6′), 130.0 (C-6), 129.3 (C-6″), 128.2 (C-2″), 127.6 (C-5″), 127.1 (C-7a), 126.2 (C-7), 125.1 (C-4), 116.0 (C-3′/C-5′), 90.1 (C-3), 28.19 (C-8), 22.2 (C-9/C-10).

3-(2,4-Dihydroxyphenyl)-3-(4-isopropylphenyl)phthalide (**5a**)

IR (KBr, v, cm^{-1}): 3368 (OH), 2962 (aliphatic C–H), 1740 (lactonic >C=O), 1610, 1590, 1508, 1465 (Ar C=C), 1255, 1113 (C–O), 1018 (C–O–C), 750, 694 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 208, 285, 320 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.1 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.9 (sept, 1H, –CH<), 6.1 and 6.2 (each s, 1H, 2× OH), 7.1–7.85 (m, 11H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 171.0 (C-1), 157.5 (C-4′), 156.7 (C-2′), 156.2 (C-3a), 148.2 (C-4″), 141.1 (C-1″), 135.1 (C-5), 131.0 (C-2″/C-6″), 130.1 (C-7), 128.2 (C-4), 128.0 (C-6′), 126.9 (C-7a), 126.5 (C-6), 125.9 (C-3″/C-5″), 113.6 (C-1′), 110.0 (C-5′), 103.9 (C-3′), 81.1 (C-3), 35.9 (C-8), 24.5 (C-9/C-10); MS (70 eV) *m/z* (%): 359 (M-1, 10), 342 (2), 317 (100), 299 (6), 273 (5), 239 (5).

3-(2,4-Dihydroxyphenyl)-3- (4-isopropyl-3-nitrophenyl)phthalide (**5b**)

IR (KBr, v, cm^{-1}): 3402 (OH), 2950 (aliphatic C–H), 1740 (lactonic >C=O), 1609, 1590, 1500, 1450 (Ar C=C), 1530 (asym. N–O), 1350 (sym. N–O), 1250, 1133 (C–O), 1020 (C–O–C), 850 (C–N), 757, 690 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 215, 290, 330 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.2 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.8 (sept, 1H, –CH<), 6.15 and 6.25 (each s, 1H, 2× OH), 6.7–8.0 (m, 10H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169.2 (C-1), 158.0 (C-4′), 156.8 (C-2′), 152.1 (C-3a), 147.0 (C-3″), 142.5 (C-1″), 141.4 (C-4″), 134.2 (C-5), 133.5 (C-6′),130.2 (C-6), 129.5 (C-6″), 128.2 (C-2″), 127.8 (C-5″), 127.0 (C-7a), 126.5(C-7), 125.3 (C-4), 113.1 (C-1′), 109.5 (C-5′), 104.5 (C-3′), 90.0 (C-3), 28.2 (C-8), 22.4 (C-9/C-10); MS (70 eV) *m/z* (%): 404 (M-1, 9), 297 (9), 208 (14), 117 (66), 91 (100).

3-(3,4-Dihydroxyphenyl)-3-(4-isopropylphenyl)phthalide (**6a**)

IR (KBr, v, cm^{-1}): 3398 (OH), 2962 (aliphatic C–H), 1740 (lactonic >C=O), 1604, 1580, 1500, 1450 (Ar C=C), 1250, 1114 (C–O), 1020 (C–O-C), 745, 600 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 215, 260, 295 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.3 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 3.0 (sept, 1H, –CH<), 7.2–8.1 (m, 11H, Ar–H), 9.1 (s, 2H, 2× OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.5 (C-1), 151.3 (C-3a), 148.1 (C-4″), 147.8 (C-3′), 145.1 (C-4′), 142.0 (C-1″), 138.0 (C-1′), 133.9 (C-5),130.9 (C-2″/C-6″), 129.7 (C-7), 129.0 (C-6′), 128.0 (C-4), 126.3 (C-7a), 125.9 (C-6), 125.6 (C-3″/C-5″), 118.2 (C-5′), 115.5 (C-2′), 82.5 (C-3), 36.3 (C-8), 24.2 (C-9/C-10).

3-(3,4-Dihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide (**6b**)

IR (KBr, v, cm^{-1}): 3497 (OH), 2950 (aliphatic C–H), 1749 (lactonic >C=O), 1600, 1580, 1500, 1450 (Ar C=C), 1550 (asym. N–O), 1350 (sym. N–O), 1255, 1116 (C–O), 1020 (C–O–C), 850 (C–N), 753, 700 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 225, 280, 330 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.2 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.7 (sept, 1H, –CH<), 6.6–8.05 (m, 10H, Ar–H), 8.85 and 9.0 (each s, 1H, 2× OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169.2 (C-1), 152.4 (C-3a), 148.2 (C-3), 147.0 (C-3″), 145.8 (C-4′), 142.3 (C-1″), 141.2 (C-4″), 137.5 (C-1′), 134.2 (C-5), 130.2 (C-6), 129.5 (C-6″), 129.0 (C-6′), 128.1 (C-2″), 127.8 (C-5″), 127.1 (C-7a), 126.6 (C-7), 125.2 (C-4), 117.5 (C-5′), 115.1 (C-2′), 90.2 (C-3), 28.5 (C-8), 22.4 (C-9/C-10).

3-(2,5-Dihydroxyphenyl)-3-(4-isopropylphenyl)phthalide (**7a**)

IR (KBr, v, cm^{-1}): 3404 (OH), 2961 (aliphatic C–H), 1750 (lactonic >C=O), 1604, 1580, 1500, 1466 (Ar C=C), 1253, 1117 (C–O), 1000 (C–O–C), 764, 690 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 210, 250, 300 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.1 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.9 (sept, 1H, –CH<), 6.5 and 6.6 (each s, 1H, 2× OH), 7.0–8.1 (m, 11H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.1 (C-1), 152.0 (C-3a), 151.7 (C-5′), 148.3 (C-2′), 146.5 (C-4″), 141.2 (C-1″), 133.8 (C-5), 130.8 (C-2″/C-6″), 129.8 (C-7), 128.1 (C-4), 126.7 (C-7a), 126.1 (C-3″/C-5″), 125.7 (C-6), 122.5 (C-1′), 118.2 (C-3′), 116.0 (C-6′), 114.3 (C-4′), 82.6 (C-3), 36.0 (C-8), 24.0 (C-9/C-10).

3-(2,5-Dihydroxyphenyl)-3- (4-isopropyl-3-nitrophenyl)phthalide (**7b**)

IR (KBr, v, cm^{-1}): 3384 (OH), 2950 (aliphatic C–H), 1749 (lactonic >C=O), 1600, 1580, 1500, 1450 (Ar C=C), 1550 (asym. N–O), 1350 (sym. N–O), 1250, 1113 (C–O), 1015 (C–O-C), 850 (C–N), 753, 700 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 220, 270, 320 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.2 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.7 (sept, 1H, –CH<), 6.5–8.0 (m, 10H, Ar–H), 9.2 (s, 2H, 2× OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169.5 (C-1), 152.5 (C-3a), 150.0 (C-5′), 148.2 (C-2′), 147.1 (C-3″), 142.7 (C-1″), 141.2 (C-4″), 135.0

(C-5), 130.2 (C-6), 129.6 (C-6″), 128.1 (C-2″), 127.6 (C-5″), 127.1 (C-7a), 126.7 (C-7), 125.0 (C-4), 123.1 (C-1′), 117.5 (C-3′), 117.0 (C-6′), 115.3 (C-4′), 90.0 (C-3), 28.4 (C-8), 22.3 (C-9/C-10).

3-(2,4,6-Trihydroxyphenyl)-3-(4-isopropylphenyl)phthalide (**8a**)

IR (KBr, ν, cm^{-1}): 3392 (OH), 2960 (aliphatic C–H), 1751 (lactonic >C=O), 1609, 1580, 1500, 1465 (Ar C=C), 1250, 1158 (C–O), 1020 (C–O–C), 760, 690 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 220, 260, 325 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.0 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.8 (sept, 1H, –CH<), 5.7 (broad s, 3H, 3× OH), 7.0–8.0 (m, 10H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.4 (C-1), 158.9 (C-4′), 158.7 (C-2′/C-6′), 152.1 (C-3a), 146.9 (C-4″), 141.5 (C-1″), 135.0 (C-5), 130.5 (C-2″/C-6″), 129.6 (C-7), 128.5 (C-4), 126.5 (C-7a), 126.3 (C-6), 126.0 (C-3″/C-5″), 110.5 (C-1′), 96.1 (C-3′/C-5′), 82.5 (C-3), 36.1 (C-8), 24.3 (C-9/C-10).

3-(2,4,6-Trihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide (**8b**)

IR (KBr, ν, cm^{-1}): 3397 (OH), 2950 (aliphatic C–H), 1718 (lactonic >C=O), 1610, 1500, 1450 (Ar C=C), 1550 (asym. N–O), 1350 (sym. N–O), 1250, 1115 (C–O), 1020 (C–O–C), 840 (C–N), 754, 700 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 210, 270, 340 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.1 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 3.1 (sept, 1H, –CH<), 5.50, 5.55, 5.60 (each s, 1H, 3× OH), 7.0–8.1(m, 9H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169.5 (C-1), 158.9 (C-4′), 158.2 (C-2′/C-6′), 152.1 (C-3a), 147.1 (C-3″), 142.8 (C-1″), 141.3 (C-4″), 134.6 (C-5), 130.0 (C-6), 129.2 (C-6″), 128.3 (C-2″), 127.9 (C-5″), 127.2 (C-7a), 126.8 (C-7), 125.0 (C-4), 110.3 (C-1′), 97.1 (C-3′/C-5′), 90.1 (C-3), 28.6 (C-8), 22.3 (C-9/C-10).

3-(2,3,4-Trihydroxyphenyl)-3-(4-isopropylphenyl)phthalide (**9a**)

IR (KBr, ν, cm^{-1}): 3422 (OH), 2962 (aliphatic C–H), 1751 (lactonic >C=O), 1600, 1575, 1500, 1466 (Ar C=C), 1250, 1112 (C–O), 1020 (C–O–C), 764, 690 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 210, 245, 290 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.0 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.8 (sept, 1H, –CH<), 6.1, 6.2, 6.3 (each s, 1H, 3× OH), 6.8–8.1 (m, 10H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.0 (C-1), 153.2 (C-3a), 148.9 (C-4′), 148.7 (C-2′), 146.8 (C-4″), 145.0 (C-3′), 140.9 (C-1″), 135.1 (C-5), 130.7 (C-2″/C-6″), 130.2 (C-7), 128.5 (C-4), 126.9 (C-7a), 126.3 (C-6), 126.2 (C-3″/C-5″), 120.0 (C-6′), 113.8 (C-1′), 110.5 (C-5′), 84.9 (C-3), 36.2 (C-8), 24.3 (C-9/C-10).

3-(2,3,4-Trihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide (**9b**)

IR (KBr, ν, cm^{-1}): 3421 (OH), 2950 (aliphatic C–H), 1750 (lactonic >C=O), 1600, 1580, 1500, 1450 (Ar C=C), 1550 (asym. N–O), 1330 (sym. N–O), 1250, 1108 (C–O), 1020 (C–O–C), 850 (C–N), 752, 690 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 215, 260, 310 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.2 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.8 (sept, 1H, –CH<), 6.2-8.5 (m, 9H, Ar–H), 9.5 (broad s, 3H, 3× OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 169.8 (C-1), 152.3 (C-3a), 150.4 (C-2′), 150.2 (C-4′), 146.8 (C-3″), 143.0 (C-1″), 141.3 (C-4″), 140.1 (C-3′), 134.5 (C-5), 134.0 (C-6′), 131.7 (C-6″), 130.0 (C-6), 128.2 (C-2″), 127.6 (C-5″), 127.0 (C-7a), 126.1 (C-7), 125.4 (C-4), 115.1 (C-1′), 108.2 (C-5′), 88.5 (C-3), 28.6 (C-8), 22.5 (C-9/C-10).

Acetylation of phthalide **5a** and **5b**: Synthesis of 3-(2,4-diacetoxyphenyl)-3-(4-isopropylphenyl)phthalide (**10a**) and 3-(2,4-diacetoxyphenyl)-3- (4-isopropyl-3-nitrophenyl)phthalide (**10b**)

A mixture of the phthalide, **5a** or **5b** (1 g), acetic anhydride (20 mL) and fused sodium acetate (3.0 g) was refluxed at 130–140 °C to get a diacetyl derivative **10a** as a pale yellow solid (0.8 g) and **10b** as a light brown solid (0.9 g). These were purified by recrystallization from acetone. The analytical and spectral data of the acetyl derivatives are given below.

10a: m.p. 152–153 °C; % yield: 65; IR (KBr, ν, cm^{-1}): IR (KBr, ν, cm^{-1}): 2960 (aliphatic C–H), 1750, 1760 (lactone and acetoxyl >C=O), 1600, 1585, 1500, 1460 (Ar C=C), 1250, 1115 (C–O), 1025 (C–O–C), 760, 700 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 210, 270, 285 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.0 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.4 (s, 6H, acetoxyl), 2.9 (sept, 1H, –CH<), 6.9–8.1 (m, 11H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.3 (C-1), 169.2 (CO, acetoxyl), 153.8 (C-3a), 150.6 (C-2′), 150.1 (C-4′), 146.2 (C-6″), 140.8 (C-1″), 135.2 (C-5), 131.2 (C-2″/C-6″), 130.5 (C-7), 128.3 (C-4), 128.1 (C-6′), 126.8 (C-7a), 126.1 (C-3″/C-5″), 125.9 (C-6), 123.5 (C-1′), 119.0 (C-5′), 115.8 (C-3′), 84.3 (C-3), 36.2 (C-8), 24.4 (C-8/C-9), 20.8 (–CH_3, acetoxyl); Anal. calcd. for $C_{27}H_{24}O_6$: C.72.96; H, 5.44. Found: C, 73.02; H, 5.58 %.

10b: m.p. 120–122 °C; % yield: 75; IR (KBr, ν, cm^{-1}): 2960 (aliphatic C–H), 1750, 1755 (lactone and acetoxyl >C=O), 1605, 1585, 1510, 1460 (Ar C=C), 1535 (asym. N–O), 1350 (sym. N–O), 1260, 1115 (C–O), 1020 (C–O–C),

840 (C–N), 760, 700 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 210, 280, 310 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.0 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.2 (s, 6H, acetoxyl), 3.2 (sept, 1H, –CH<), 7.5–8.15 (m, 10H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.0 (C-1), 168.0 (CO, acetoxyl), 153.0 (C-3a), 150.1 (C-2′), 149.5 (C-4′), 146.8 (C-3″), 142.1 (C-1″), 141.5 (C-4″), 134.6 (C-5), 129.8 (C-6), 129.4 (C-6′), 129.2 (C-6″), 128.1 (C-2″), 127.8 (C-5″), 127.1 (C-7a), 126.2 (C-7), 125.4 (C-4), 123.0 (C-1′), 118.5 (C-5′), 115.5 (C-3′), 88.5 (C-3), 28.5 (C-8), 22.5 (C-9/C-10), 20.5 (–CH_3, acetoxyl); Anal. calcd. for $C_{27}H_{23}NO_8$: C, 66.25; H, 4.74; N, 2.86 Found: C, 66.55; H, 4.76; N, 2.89 %.

Bromination of phthalide **5a** and **5b**: Synthesis of 3-(3,5-dibromo-2,4-dihydroxyphenyl)-3-(4-isopropylphenyl)phthalide (**11a**) and 3-(3,5-dibromo-2,4-dihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide (**11b**)

The phthalide **5a** or **5b** (1.0 g) was dissolved in ethanol (10 mL) and to the resulting solution, an excess of bromine (2 mL) was added gradually with constant shaking. The contents were left overnight. The deposited solid mass was thoroughly washed with water and crystallized from acetic acid to get dibromo compounds **11a** as a brick-red powder (1.2 g) and **11b** as a brownish orange powder (1.0 g). The analytical and spectral data of the synthesized dibromo derivatives are given below.

11a: m.p. 280–282 °C; % yield: 86; IR (KBr, ν, cm^{-1}): 3250 (OH), 2960 (aliphatic C–H), 1760 (lactonic >C=O), 1605, 1590, 1500, 1460 (Ar C=C), 1260, 1120 (C–O), 1010 (C–O–C), 755, 695 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 215, 260, 335 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.1 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 3.0 (sept, 1H, –CH<), 6.9–7.9 (m, 12H, Ar–H), 9.5 (broad s, 2H, 2× OH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.9 (C-1), 153.4 (C-3a), 153.0 (C-2′), 152.1 (C-4′), 146.3 (C-6″), 141.6 (C-1″), 135.6 (C-5), 131.4 (C-2″/C-6″), 131.0 (C-6′), 130.0 (C-7), 128.1 (C-4), 126.7 (C-3″/C-5″), 126.3 (C-7a), 126.2 (C-6), 117.5 (C-1′), 109.8 (C-5′), 109.0 (C-3′) 84.9 (C-3), 36.1 (C-8), 23.5 (C-9/C-10); Anal. calcd. for $C_{23}H_{18}O_4Br_2$: C, 53.31; H, 3.50; Br, 30.84. Found C, 53.52; H, 3.48; Br, 30.69 %.

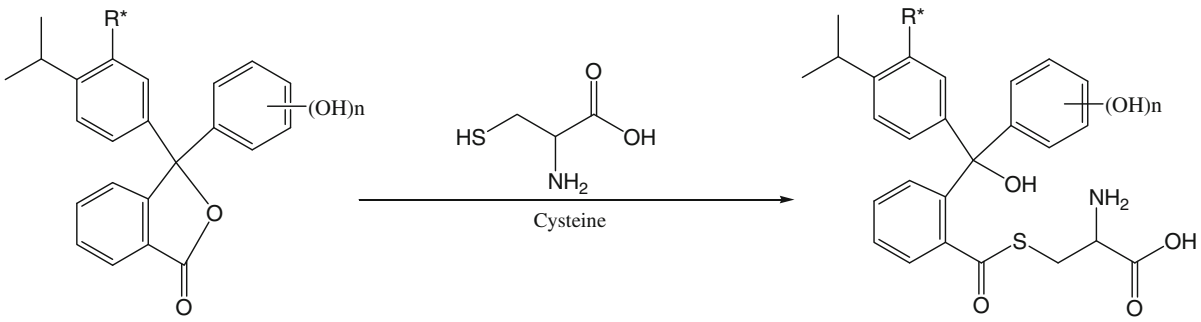
11b: m.p. 288–290 °C; % yield: 72; IR (KBr, ν, cm^{-1}): 3300 (OH), 2955 (aliphatic C–H), 1760 (lactonic >C=O), 1610, 1590, 1500, 1460 (Ar C=C), 1530 (asym. N–O), 1340 (sym. N–O), 1260, 1110 (C–O), 1020 (C–O–C), 845 (C–N), 758, 695 (o-disubstituted phthalide ring); UV (methanol): λ_{max} 220, 290, 340 nm; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.1 (d, 6H, 2× CH_3, $J = 6.5$ Hz), 2.5 (sept, 1H, –CH<), 6.1(broad s, 1H, 2× OH), 7.0–8.1 (m, 8H, Ar–H); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm): 170.0 (C-1), 153.2 (C-3a), 153.1 (C-2′), 151.8 (C-4′), 146.9 (C-5″), 142.1 (C-1″), 141.5 (C-4″), 135.2 (C-5), 132.2 (C-6″), 130.0 (C-6), 129.4 (C-2″), 128.1 (C-6″), 127.2 (C-3″), 127.1 (C-7a), 126.4 (C-7), 125.7 (C-4), 118.0 (C-1′), 110.5 (C-5′), 109.2 (C-3′), 86.0 (C-3), 28.6 (C-8), 22.3 (C-9/C-10); Anal. calcd. for $C_{23}H_{17}Br_2NO_6$: C, 49.05; H, 3.04; Br, 28.3; N, 2.49. Found C, 49.11; H, 3.08; Br, 28.7; N, 2.52 %.

Caustic potash fusion of **5a** and **5b**

The phthalide **5a** or **5b** (1 g) was mixed with a paste of KOH pellets (10 g) in water and the mixture was strongly heated for 1.5 h. The fused mass was cooled, dissolved in water and filtered. The filtrate was acidified with dil. HCl, when a solid (**A**) was obtained. It was filtered and the filtrate was shaken with ether. Evaporation of the ether produced another solid (**B**). The solid **A** was identified as 2-(4-isopropylbenzoyl)benzoic acid (**1a**) or 2-(4-isopropyl-3-nitrobenzoyl)benzoic acid (**1b**), and the solid **B** was identified as resorcinol by direct comparison (m.m.p., co-TLC and superimposable IR spectra) with their authentic samples.

Biological activity

The synthesized phthalides (**4a–11a** and **4b–11b**) are expected to exhibit a large array of biological properties. During the present study we have evaluated a few phthalides for their antibacterial and antifungal activities. The phthalides 3-(3,4-dihydroxyphenyl)-3-(4-isopropylphenyl)phthalide (**6a**), 3-(3,4-dihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide (**6b**), 3-(2,3,4-trihydroxyphenyl)-3-(4-isopropylphenyl) phthalide (**9a**) and 3-(2,3,4-trihydroxyphenyl)-3-(4-isopropyl-3-nitrophenyl)phthalide (**9b**) were tested for their in vitro antibacterial activity against *Staphylococcus aureus* ATCC 29213 (Sa), methicillin-resistant *S. aureus* ATCC 33591 (MRS), *Escherichia coli* ATCC 35218 (Ec), *Pseudomonas aeruginosa* ATCC 27853 (Pa) and *Mycobacterium intracellulare* ATCC 23068 (Mi). The in vitro antifungal activity of the phthalides **6a, 6b, 9a** and **9b** was determined against *Candida albicans* ATCC 90028 (Ca), *Candida glabrata* ATCC 90030 (Cg), *Candida krusei* ATCC 6258 (Ck), *Cryptococcus neoformans* ATCC 90113 (Cn) and *Aspergillus fumigatus* ATCC 204305 (Af). The compounds selected for the antimicrobial screening contain catechol and pyrogallol structural units in their structures. The rationale to select these compounds is the fact that catechol and pyrogallol are allelo chemicals belonging to phenolic compounds synthesized in plants (Kocaçalışkan *et al*., 2006), and significant antibacterial activity is associated with them (Zhang, 2005, 2000; Zhang et al., 2003a, b; Foti *et al*., 2002; Hussain *et al*., 2003; Chichirau *et al*., 2005; Flueraru *et al*., 2005).



Phthalide (**4-11**) Phthalide - cysteine adduct

Scheme 4 Possible formation of phthalide–cysteine adduct

All organisms were tested using modified versions of the CLSI (formerly NCCLS) methods. For all organisms excluding *M. intracellulare* and *A. fumigatus*, optical density was used to monitor the growth (NCCLS, 2002a, b; NCCLS, 2006). Media supplemented with 5 % Alamar Blue™ (BioSource International, Camarillo, CA) was utilized for growth detection of *M. intracellulare* (NCCLS, 2003; Franzblau *et al*., 1998) and *A. fumigatus* (NCCLS, 2002a, b). Samples (dissolved in DMSO) were serially-diluted in 20 % DMSO/saline and transferred (10 µL) in duplicate to 96 well flat bottom microplates. Inocula were prepared by correcting the OD_{630} of microbe suspensions in incubation broth [RPMI 1640/0.2 % dextrose/0.03 % glutamine/MOPS at pH 6.0 (Cellgro) for *Candida* spp., Sabouraud Dextrose for *C. neoformans*, cation-adjusted Mueller–Hinton (Difco) at pH 7.3 for *Staphylococcus* spp., *E. coli*, and *P. aeruginosa*, 5 % Alamar Blue™(BioSource International, Camarillo, CA) in Middlebrook 7H9 broth with OADC enrichment, pH 7.0 for *M. intracellulare*, and 5 % Alamar Blue™/RPMI 1640 broth (0.2 % dextrose, 0.03 % glutamine, buffered with 0.165 M MOPS at pH 7.0) for *A. fumigatus* to afford an assay volume of 200 µL and final target inocula of *Candida* spp. and *C. neoformans*: 1.5 × 103; *M. intracellulare*: 2.0 × 106; *Staphylococcus* spp., *E. coli*, *P. aeruginosa*: 5.0 × 105 CFU mL^{-1} and *A. fumigatus*: 2.7 × 104 CFU mL^{-1}. Final sample test concentrations are 1/100th the DMSO stock concentration.

Drug controls [Ciprofloxacin (ICN Biomedicals, Ohio) for bacteria and Amphotericin B (ICN Biomedicals, Ohio) for fungi] are included in each assay. All organisms are read at either 530 nm using the Biotek Powerwave XS plate reader (Bio-Tek Instruments, Vermont) or 544 ex/590 em, (*M. intracellulare, A. fumigatus*) using the Polarstar Galaxy Plate Reader (BMG LabTechnologies, Germany) prior to and after incubation *Candida* spp. at 35 °C for 46–50 h; *Staphylococcus* spp., *E. coli* and *P. aeruginosa* at 35 °C for 16–20 h; *C. neoformans* at 35 °C for 70–74 h; *A. fumigatus* at 35 °C for 46–50 h and *M. intracellulare* at 37 °C and 10 % CO_2 for 70–74 h. IC_{50} (concentrations that afford 50 % inhibition relative to controls) were calculated using XLfit 4.2 software (IDBS, Alameda, CA) using fit model 201. The MIC was defined as the lowest test concentration that allows no detectable growth (for *M. intracellulare* and *A. fumigatus*, no colour change from blue to pink). Results of antimicrobial screening of compounds are given in Tables 2 and 3.

Mode of action of phthalides on biological systems

Despite of the extensive studies on biological activity of phthalides, very little attention has been paid towards their mode of action. However, it has been suggested that the presence of five membered lactone ring in phthalides is responsible for their bioactivity (Beck and Chou, 2007). The amino acid cysteine, and certain enzymes (containing mercapto groups) play an important role in growth and normal activity of bacteria and other microorganisms. On the basis of an experiment involving the action of butylidenephthalide on hairless mouse, Sekia *et al.* (2000) showed the formation of butylidenephthalide–cysteine adduct as a urinary metabolite. This work suggested that occurrence of this reaction in bacteria inhibits their growth and activity. Examination of the structures of the various phthalides (**4–11**) synthesized in the present study indicates their ability to react with cysteine to form adducts (Scheme 4) and show antimicrobial activity.

Conclusion

New 3-substituted phthalides of biological interest are synthesized in 50–98.6 % yield by the condensation of 2-(4- isopropylbenzoyl)benzoic acid and 2-(4-isopropyl-3-nitrobenzoyl)benzoic acid with phenols without the use of a solvent. The procedure is simple, convenient, efficient, environmentally benign and economic as it involves the use of easily available cheap chemicals.

All the tested compounds (**6a**, **6b**, **9a** and **9b**) have shown promising antibacterial activity especially against *S. aureus* ATCC 29213 and methicillin-resistant *S. aureus* ATCC 33591 (as can be seen from Tables 2, 3). Compound **9b** also showed promising antifungal activity against *C. glabrata* ATCC 90030 (Table 3). Although, the phthalides taken for in vitro antimicrobial screening were found to be much less active than the Ciprofloxacin and Amphotericin B, which were used as control drugs. But in view of growing incidences of drug resistance in microorganisms, and remarkable bioenhancing property exhibited by some less or non-active compounds, further appropriate investigations of anticipated biological properties (including in vivo antimicrobial screening) of these phthalides are warranted to explore their significant therapeutic potential. It is well known that in vivo assessment is necessary even for drugs with little or no activity under best in vitro conditions (Emmons *et al*., 1977).

The anticancer and antioxidant properties of these compounds are under investigation and results of this ongoing work will be communicated in near future. The phthalides reported in this paper can be evaluated for other possible biological properties as well as used as lead molecules for the development of new drugs.

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