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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

I_2 catalyzed Friedel–Crafts alkylation reaction of substituted anilines with ninhydrin: formation of novel products and their antimicrobial evaluation

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Abstract Friedel–Crafts reaction of differently substituted anilines with ninhydrin in the presence of molecular iodine at ambient temperature constitutes a facile, cost effective, and regioselective synthesis of a series of 2-mono/2,2-bis-(amino-phenyl)-indane-1,3-dione derivatives. Under identical conditions, use of different substituents in aniline ring led to the formation of different products, emphasizing the pivotal role of the nature and position of the substituents in product formation. In vitro antimicrobial activity evaluation of the synthesized molecules against eight bacterial and four fungal strains revealed that four of them possess duel efficacies (bactericidal as well as fungicidal). The activities are attributed to the disruption of architecture of the microbes as revealed from ultrastructural studies (SEM).

Keywords Friedel–Crafts reaction · Ninhydrin · Molecular iodine · 2,2-bis-(amino-phenyl)-indane-1,3-dione · Regioselectivity

Introduction

Diaryl derivatives of cyclic ketones like ninhydrin, alloxan, isatin, and parabanic acid have been studied extensively due to their interesting biological activities, namely, antibacterial, antiprotozoal, anti-inflammatory, anticonvulsant, anticancer, laxative, and diuretic properties (Garrido *et al*., 1975; Poupelin *et al*., 1980; Shoichet *et al*., 1993).

In most of the cases, the derivatives were prepared by Friedel–Crafts type reactions employed on different arenes, catalyzed by an inorganic acid or a Lewis acid, where excess or stoichiometric amounts of catalyst and prolonged heating were required for the completion of the reactions (Klumpp *et al*., 1998, 1999; Song *et al*., 1998). The hazardous reaction conditions and the use of excess amount of Lewis or inorganic acids meant that these reactions were neither eco-friendly nor atom economic. The demanding environmental legislation and public/corporate pressure toward cleaner technology (Anastas and Warner, 1998; Dallinger and Kappe, 2007; Polshettiwar and Varma, 2007) has driven organic chemists to search for alternatives to acid-dependent organic reactions. Great efforts have been made to discover and optimize new reactions/methodologies which would be operationally simple, cost effective, atom economic as well as environmentally benign. As a part of this effort, very recently we have developed a protocol for the synthesis of novel bis-indolylindane-1,3-diones from ninhydrin and 3-substituted/unsubstituted indoles (Naskar *et al*., 2010). Ninhydrin and electron-rich anilines/aromatic amines are versatile reagents, the former possessing several potential electrophilic sites and the latter multiple nucleophilic sites (Taylor and Joulié, 1998). The acid-catalyzed reactions of ninhydrin with electron-rich aromatic amines have yielded various products (Fig. 1), the regiochemistry of which remains to be well explained (Bullington and Dodd, 1993; Black *et al*., 1994; Friedman, 1967). Chatterjee and Shapiro reported (Shapiro and Chatterjie, 1970) that anilines with an electron-releasing group at the meta-position react with ninhydrin, initially

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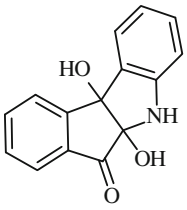
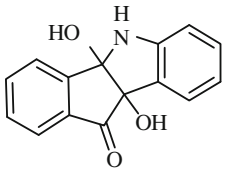
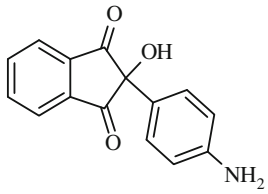
Fig. 1 Products of ninhydrin with aromatic amines



1a



1b



1c

through nitrogen, to give tetrahydroindeno[2,1-*b*]indolones (**la**). Bullington and Dodd first demonstrated that the reaction of ninhydrin with substituted anilines could afford tetrahydroindeno[1,2-*b*]indolones (**1b**), and suggested that the initial attack was not by the amino function but by the activated carbon atom at the ortho-position (Bullington and Dodd, 1993). Black *et al*. reported that treatment of ninhydrin with a number of substituted electron-rich anilines under anhydrous condition provided *o*-substituted indolones (**1b**) as the major products. However, the *p*-substituted derivatives (**1c**) were the predominant products in aqueous solvent (Black *et al*., 1994). These interesting results intrigued us to pursue a thorough study of the reaction of ninhydrin with substituted anilines.

We intended to carry out reactions in the presence of molecular iodine, because the reagent has received considerable attention as an inexpensive, nontoxic, and readily available catalyst for various organic transformations with excellent yields and high degree of selectivity (Yadav *et al*., 2007; Kidwai *et al*., 2007; Togo and Iida, 2006). We also investigated the effect of the substitution in the formation of the products and the suitability of I_2 over other Lewis acid catalysts for this reaction. In this communication, we wish to report that reacting ninhydrin with anilines in the presence of molecular iodine at ambient temperature constitutes an easy and straight forward synthetic route to 2-monosubstituted/2,2-disubstituted-1,3-indanediones. Finally, the microbicidal efficacy of the synthesized analogs has also been studied.

Results and discussion

Chemistry

In our initial experiment, ninhydrin (**1**) and aniline (**2a**) were chosen as the model reaction partners to study the reactions under various catalytic conditions. In each case, two equivalents of aniline were added to the mixture of one equivalent of ninhydrin and 5 mol% of the catalyst. Iodine, various other Lewis acid catalysts, and different solvents were screened for the standardization of the reaction. With molecular iodine as catalyst in acetonitrile, the reaction was found to be complete (TLC) within 6 h. Usual workup followed by chromatographic separation afforded a brown-colored crystalline product in very good yield. Evaluation of ^{1}H and ^{13}C NMR spectral data in DMSO-d_6 and mass spectral analysis clearly revealed that two aniline units have coupled at the C_2 of ninhydrin to form **3a**. In ^{1}H NMR, the presence of a distinct two proton singlet at δ 5.14 clearly indicated that the coupling did not involve the NH_2 group of aniline. No shifting of equilibrium toward ninhydrin and aniline on D_2O addition also indicative that the reaction had indeed taken place on the aromatic ring (Bullington and Dodd, 1993). For unambiguous determination of the structure, single crystal X-ray analysis was performed, which confirmed the structure of the product as 2,2-bis-(4-amino-phenyl)-indan-1,3-dione, **3a** (Fig. 2). The formation of such a diarylated derivative of ninhydrin with aniline is unprecedented under mild reaction conditions as employed by us, although in super acid medium ninhydrin produced similar products with substituted benzenes (Klumpp *et al*., 1998 and Song *et al*., 1998).

The reactions were also performed using different Lewis acids like $AlCl_3$, $ZnCl_2$, CAN, $FeCl_3$, $SnCl_4$, and montmorollonite K10 in CH_3CN (Table 1). Molecular iodine

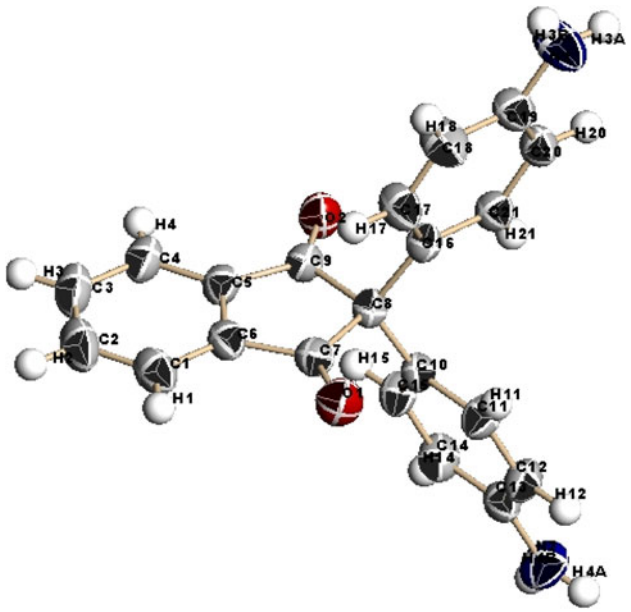


Fig. 2 ORTEP representations of the title compound **3a** the displacement ellipsoids are drawn at a probability of 50 %

Table 1 Reaction of ninhydrin (**1**) and aniline (**2a**) using different Lewis acid catalysts

Entry	Catalyst	Time (h)[a]	Isolated yield[b] (%)
1	I_2	6	90
2	$FeCl_3$	6	35
3	$SnCl_4$	8	45
4	$ZnCl_2$	7	45
5	$InCl_3$	9	20
6	Ceric (IV) ammonium nitrate	8	40
7	Montmorillonite K-10 clay	10	55
8	I_2	10	90

Reaction conditions: All the reactions were performed in acetonitrile solvent at rt

[a] Extension of the reaction time did not improve yield of the product

[b] Isolated yield of **3a**

Table 2 Standardization of the reaction conditions for the reaction of ninhydrin (1) and aniline (2**a**) using different solvents

Entry No	Solvent	Time (h)[a]	Isolated yield[b] (%)
1	CH_3CN	1	90
2	CH_2Cl_2	6	35
3	Toluene	8	15
4	CH_3NO_2	6	25
6	THF	5	58
7	^{i}PrOH	4	86
8	MeOH	6	75

[a] *Reaction Conditions*: Ninhydrin (1.68 m mol), aniline (3.36 m mol), iodine (5 m mol% w.r.t ninhydrin), rt

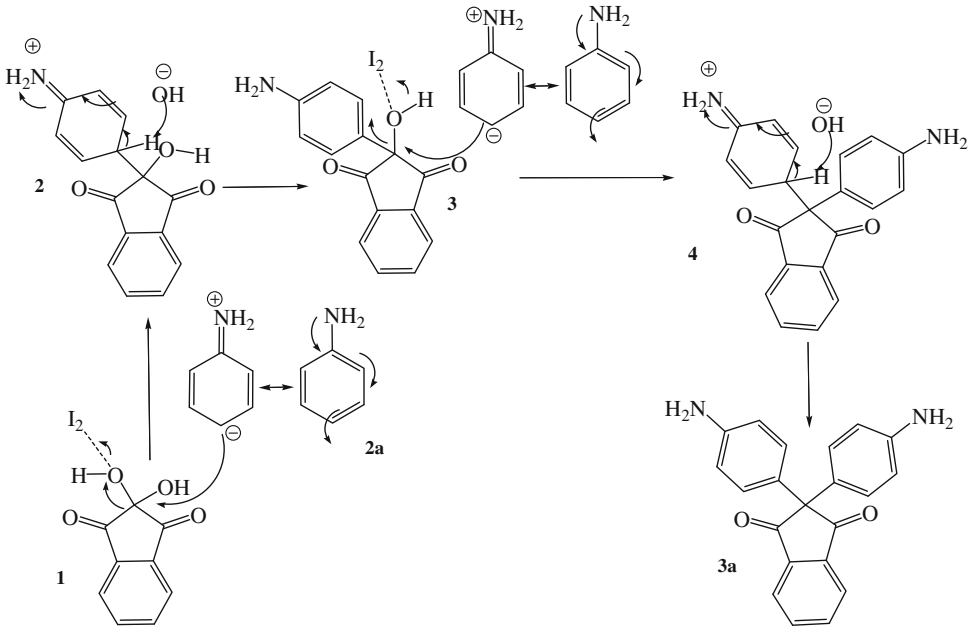
[b] Isolated yield

appears to produce the best result. However, no significant increase in yield was observed with the increase of catalyst loading (from 5 mol%) or of the reaction time, though reaction carried out in the absence of iodine did not yield any product even after 24 h.

Following the same reaction protocol, the study was carried out in other solvents, viz. CH_2Cl_2, CH_3NO_2, CH_3OH, DMF, THF, and ^{i}PrOH. The results (Table 2) reveal that the reactions were more effective in polar solvents compared to non polar ones with respect to yield of the product as well as the reaction time. Acetonitrile emerged to be the most effective solvent and therefore all the remaining studies were performed in this medium.

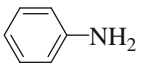
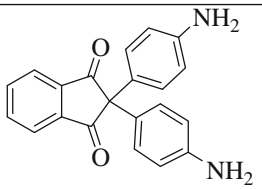
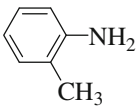
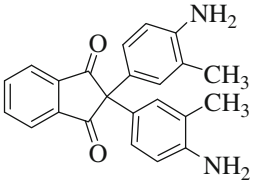
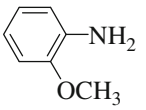
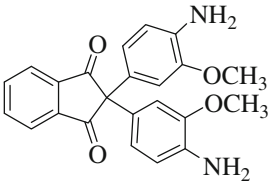
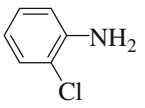
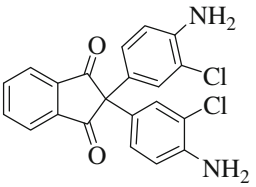
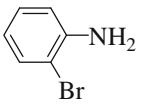
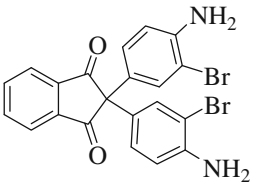
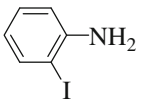
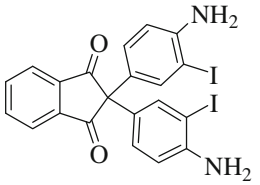
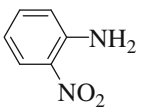
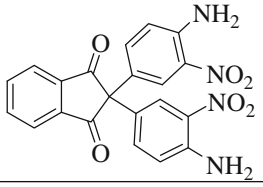
We then performed reactions using differently substituted anilines both for the generalization of this methodology and for studying the effect of substitutions (electron donating or withdrawing) at different positions of the aniline ring (Scheme 1). The reactions performed with *N*-substituted anilines revealed that *N*-methyl aniline (**2j**) produced an analogous product **3j** (Table 3,entry 10) in higher yield, but *N*,*N*-dimethyl (**2n**) and diethyl aniline (**2o**) produced only monoalkylated products (**3n** and **3o**) even after stirring for 24 h at room temperature (entry 14,15). Similar type of product [2-(5-amino-quinolin-8-yl)-2-hydroxy-indan-1,3-dione), **3q**] was also obtained when 5-aminoquinoline (**2q**) was used as the nucleophile (entry 17). It may be mentioned that monoarylations at C-2 position of ninhydrin have been reported in the literature with substituted anilines, though under different reaction conditions (Taylor and Joulié, 1998; Bullington and Dodd, 1993; Black *et al*., 1994).

Next, we attempted reactions with anilines substituted at different positions of the ring. In case of electron-rich



Scheme 1 Plausible pathway for the formation of symmetrical 2,2-bis-(4-amino-phenyl)-indane-1,3-diones

Table 3 Reaction of ninhydrine (1) and differently substituted aniline (**2a–q**)[a]

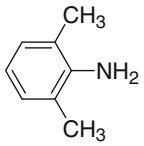
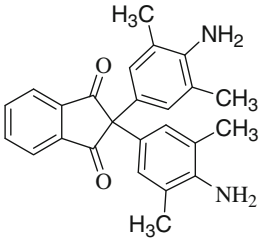
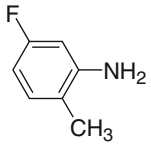
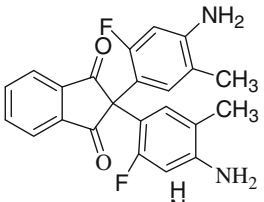
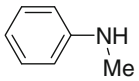
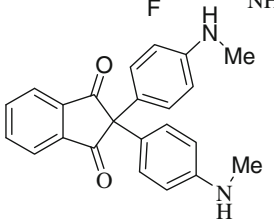
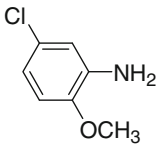
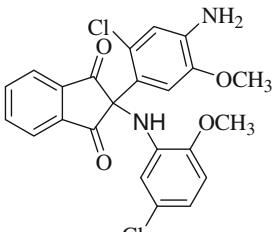
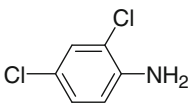
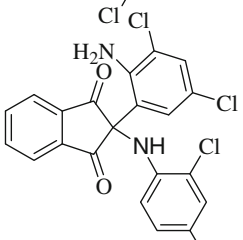
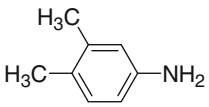
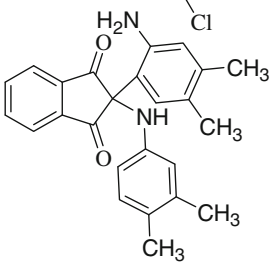
Entry No.	Alkylating Agent	Time (h)	Product[b]		Yield[c] (%)
1		6		**3a**	80
2		7		**3b**	75
3		5		**3c**	80
4		9		**3d**	84
5		6		**3e**	75
6		5		**3f**	78
7		7		**3g**	80

anilines, diarylated products were isolated with higher yields than with deactivated anilines. But 2,6-dimethyl aniline (**2h**), which is electron rich, also afforded slightly lower yield of the product, ostensibly due to steric inhibition of resonance by the two methyl groups (entry 8).

Finally, we studied the effect of substituents located at two different positions of the aniline ring.

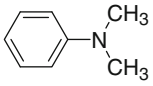
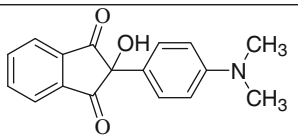
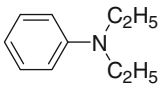
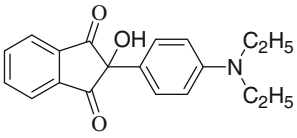
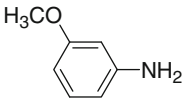
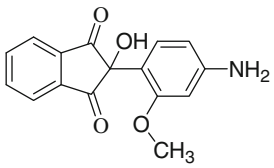
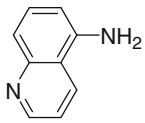
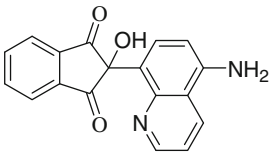
The results permit the following conclusions to be drawn: (i) *N*-dialkylated anilines (entries 14, 15) react through the para-position (both had a free para carbon atom) to form monosubstituted products. (ii) *N*-unsubstituted or monosubstituted derivatives with free para-position (entries 1–11) afford disubstituted products. It seems likely that the intermediate monosubstituted product

Table 3 continued

Entry No.	Alkylating Agent	Time	Products		Yield(%)
8		6		**3h**	70
9		7		**3i**	65
10		6		**3j**	90
11		7		**3k**	84
12		6		**3l**	75
13		5		**3m**	80

suffers dehydration to generate a quinone imine type compound, which readily undergoes nucleophilic addition to form the final disubstituted product. If the meta carbon in the aniline ring carries a bulky group (entry 11), the second aniline molecule prefers to attack through the nitrogen atom to avoid a sterically encumbered product, leading to an unsymmetrically disubstituted derivative (**3k**) (Scheme 2). The only exceptions are m-methoxyaniline (entry 16) and the quinoline derivative (entry 17), where the reaction proceeds only to monosubstitution despite the presence of an NH_2 group. Perhaps the m-substituent (methoxy or ring residue) in these cases is large enough to

Table 3 continued

Entry No.	Alkylating Agent	Time	Products		Yield(%)
14		9		**3n**	78
15		7		**3o**	80
16		6		**3p**	72
17		8		**3q**	68

[a] All the reactions were carried out in acetonitrile using 5 mol% of iodine as catalyst at rt

[b] All the products were characterized by mass, ^{1}H, ^{13}C NMR

[c] Isolated yield

discourage the dehydration step. (iii) Anilines having a substituted para-position (entries 12 and 13) react through the ortho carbon, and through the less crowded one if there be a choice. The second aniline attacks via the nitrogen atom thereafter, as the symmetrically substituted product would have been sterically crowded (**3l**, **m**) (Scheme 2).

Attempts were also made to isolate the intermediate to the unsymmetrically disubstituted products to ascertain which of the groups, the amino or activated carbon, gets coupled first with ninhydrin. A series of reactions were performed following the aforesaid protocols and monitoring at different time intervals (30 min and 60 min). However, in all cases the products isolated were the disubstituted ones. Reactions performed with one equivalent of aniline and monitored at different time intervals also failed to furnish any monosubstituted product. Instead, we could isolate only the disubstituted product (yields proportional to the time) and the unutilized ninhydrin.

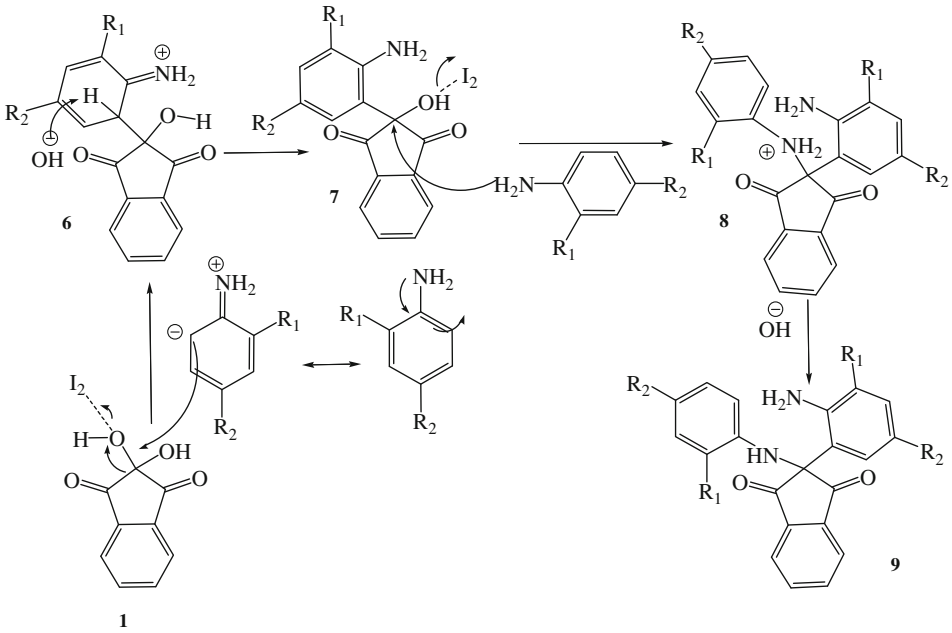
In order to make the methodology an environmentally benign one, we tried to perform few reactions with differently substituted anilines and ninhydrin in water in the presence of molecular iodine with KI. It is noteworthy that within 15 min of the reaction, the solid products precipitate out of the solution. These were characterized as the monoarylated products as reported in the literature (Bullington and Dodd, 1993, Kok and Roth, 1975; Roth and Kok, 1976. The monoarylated products (**3n–q**) were further reacted with unhindered anilines but failed to produce the corresponding biarylated product.

We next tried to prepare mixed type biarylated products using different aniline units. For this purpose, a mixture of two differently substituted anilines, viz. 2-methoxyaniline (**2c**) and 2-bromoaniline (**2e**), one equivalent each with respect to ninhydrin, were used. Interestingly, the products were the symmetrically diarylated ones (**3c**, **3e**) without any cross product. Similar result was also obtained when we used the anilines one after another.

All the products obtained from the reactions were purified by chromatography and are quite stable. It is noteworthy that our attempts to rearrange the monoarylated product (**3n–q**) in acetic acid medium (Bullington and Dodd, 1993) also failed.

The structures were determined with the help of ^{1}H and ^{13}C NMR data along with extensive 2D NMR spectrum analysis. Important correlations of COSY, HMBC, and NOESY of a representative compound **3k** are being presented in Fig. 3. The proposed structure of **3k** was supported by an unambiguous single crystal X-ray diffraction analysis (Fig. 4).

Scheme 2 Plausible pathway for the formation of unsymmetrical 2-(2-amino-4,5-dimethyl-phenyl)-2-(3,4-dimethyl-phenylamino)-indan-1,3-dione



Antimicrobial activity

The synthesized compounds were tested in vitro for their antibacterial and antifungal effects. The microorganisms used in this study consisted of eight strains of bacteria, namely, *Bacillus pumilus 11778*, *Staphylococcus aureus* 29737, *Micrococcus luteus* ATCC 9341, *Escherichia coli* 319, *Klebsiella pneumoniae J/I/4*, *Pseudomonas aeruginosa* 71, *Vibrio cholerae* 759, and *Shigella dysenteriae* 15. Antifungal studies were carried out on four strains of fungi, viz. *Aspergillus niger*, *Candida albicans*, *C. tropicalis*, and *Cryptpcoccus neoformans*. The results of the antimicrobial activity study given in Table 4 demonstrate that among the tested compounds, nine possess a good degree of antibacterial activity against various bacterial strains. The MIC tests revealed that **3k** had MIC value 32 μg/ml against two bacterial strains (*Micrococcus luteus ATCC 9341* and *Vibrio cholerae 759*) and their zone diameter were found within 8–9 mm; **3d** also had very good activity against three stains (*S. dysenteriae* 15, *E. coli* 319, *K. pneumoniae J/I/4*) with MIC value between 32 and 128 μg/ml and zone diameter within 6–8 mm. Similarly, both **3i** and **3j** are effective against three bacterial stain with MIC value 128 μg/ml and the bacterial stains for **3i** were *E. coli* 319, *B. pumilus 11778*, *K. pneumoniae J/I/4* and that for **3j** *E. coli* 319, *V. cholerae* 759, *K. pneumoniae J/I/4*; **3e** active against four bacterial stains (*E. coli* 319, *B. pumilus 11778*, *V. cholerae* 759, *K. pneumoniae J/I/4*) with MIC value 128 μg/ml and zone of diameter 6–9 mm. The derivatives **3f**, **3o**, and **3b** showed activity against three bacterial stains with MIC value 64–128 μg/ml where **3f** active against *S. aureus* 29737, *M. luteus* ATCC 9341, and

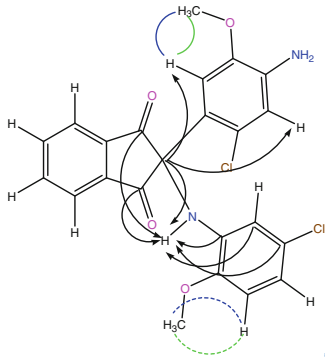


Fig. 3 Important correlations COSY (⌒) HMBC (↷) NOESY (⌒) of **3k** (Color figure online)

P. aeruginosa 71 with zone diameter of 8 mm, and **3o** and **3b** active against *S. dysenteriae* 15, *E. coli* 319, and *B. pumilus 11778* with zone diameter of 7–9 mm. **3b** is also active against *V. cholerae* 759. Likewise, **3a** and **3l**, showed good activity against four bacterial stains with MIC value 100–128 μg/ml. **3a** showed activity against *S. dysenteriae* 15, *E. coli* 319, *B. pumilus 11778*, *and K. pneumoniae J/I/4* with zone diameter 6–8 mm and **3l** showed activity against *S. dysenteriae* 15, *E. coli* 319, *V. cholerae* 759, and *K. pneumoniae J/I/4* with zone diameter 7–8 mm (Table 5).

Among the identified nine effective derivatives, seven are diarylated ones. The diarylated product with aniline (**3a**) or *o*-anisidine (**3b**) emerged effective against all eight bacterial strains with varying degree of efficacy. Products derived from ortho-substituted anilines displayed efficacy against three to eight bacterial strains, while those obtained from meta-or para-substituted ones showed insignificant effect. Interestingly, products with a methyl

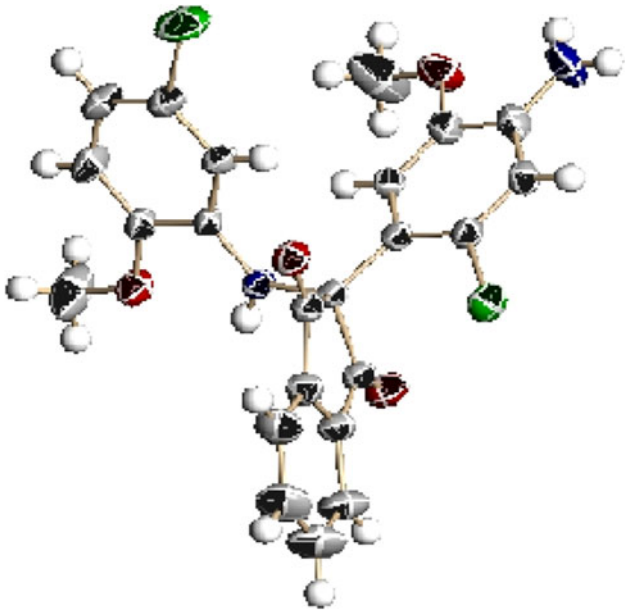


Fig. 4 ORTEP representations of the title compound **3k**, the displacement ellipsoids are drawn at a probability of 50 %

group at ortho-position showed activity against greater number of bacterial strains than halo-substituted analogs, possibly due to the electronegativity of the halogens, which might have led to unfavorable hydrophilic interaction. Likewise, substitution on NH_2 with hydrophobic group (methyl or ethyl) as in **3n** or **3o** generated sign ificant effect toward few bacterial strains, likely due to favorable hydrophobic interaction at the binding site.

A viable colony count method was performed to confirm the antimicrobial test result of the compounds and the results are shown in Figs. 5 and 6. Significantly lower levels of viable CFU count compared to control (without compound) indicated test compound (**3b**, **3k**) incorporation and bactericidal effect was noticed against Gram-negative organisms, i.e., *E. coli 319* and *Vibrio cholerae 759*.

After treatment with the test compounds, bacterial cell structure was observed by SEM micrographs (Fig. 7), which demonstrated that some morphological changes had occurred in bacteria after the treatments. In case of *E. coli* 319, the original rod shape was swollen to assume an irregular shape with high agglomeration of the cells. It is presumed that antimicrobial activity of the synthesized product may be due to damage of microbial enzyme or cell wall permeability. The antimicrobial activity of test product is also related to its ability to modify the DNA replication mechanism as well as to cause abnormalities in size, cytoplasmic contents, cell membrane, and outer cell layers of sensitive cells (Russell and Hugo, 1994). The study revealed that Gram-negative bacteria were more susceptible to the antimicrobial effects of samples than Gram-positive ones, presumably due to the thinner nature of their peptidoglycan cell wall network, which may allow more rapid absorption of the drug into the cell.

Among all the tested compounds, only four (**3a**, **3b**, **3e**, **3l**) appeared effective against three fungi, i.e., *Aspergillus niger*, *Candida albicans*, and *C. tropicalis*, which had MIC values ranging between 1,000 and 2,500 μg/ml, while **3e** showed recognizable antifungal activity against *Cryptococcus neoformans* also (Table 6). The zone of inhibition of the compounds is shown in Table 7.

Conclusion

In summary, we have developed an easy, simple, and mild reaction condition for the construction of 2,2-diarylindanediones in neutral medium by the reaction of anilines with

Table 4 Antibacterial activity of test compounds against different bacterial strains

Name of the organisms	Minimum inhibitory concentration (mg/ml) of test compounds and standard antibiotics																		
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**3h**	**3i**	**3j**	**3k**	**3l**	**3m**	**3n**	**3o**	**3p**	**3q**	**GT**	**AM**
Staphylococcus aureus 29737	>1,000	>500	_	_	_	64	64	_	_	_	_	_	_	128	_	128	_	1	0.5
Shigella dysenteriae 15	128	128	_	32	_	_	_	_	_	_	_	128	_	_	128	_	_	2	10
Micrococcus luteus ATCC 9341	>1,000	>500	_	_	_	128	_	_	_	_	32	_	_	_	_	128	_	24	1
Escherichia coli 319	128	64	_	100	128	_	_	_	128	128	_	100	128	100	64	_	100	0.5	4
Bacillus pumilus 11778	100	128	250	_	128	_	_	250	128	_	_	_	_	_	128	_	_	10	0.5
Pseudomonas aeruginosa 71	>500	>1,000	_	_	_	128	_	_	_	_	_	_	_	_	_	_	_	4	256
Vibrio cholerae 759	>500	128	250	_	128	_	_	_	_	128	32	128	_	_	_	_	_	2	256
Klebsiella pneumoniae J/I/4	128	500	250	128	128	_	_	_	128	128	_	128	_	_	_	_	_	0.25	256

GT Gentamicine, *AM* Amoxicilline

Table 5 Zone of inhibition of the tested compounds

Diameter of zone inhibition in mm																
Name of the organisms	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l	3m	3n	3o	3p	3q
Staphylococcus aureus 29737	–[a]	–	–	–	–	8 ± 0.41	8 ± 0.04	–	–	–	–	–	–	7 ± 0.02	–	7 ± 0.02	–
Shigella dysenteriae 15	7 ± 0.02	7 ± 0.02	–	6 ± 0.02	–	–	–	–	–	–	–	7 ± 0.05	–	–	7 ± 0.02	–	–
Micrococcus luteus ATCC 9341	–	–	–	–	–	8 ± 0.03	–	–	–	–	9 ± 0.05	–	–	–	–	8 ± 0.05	–
Escherichia coli 319	6 ± 0.4	7 ± 0.2	–	8 ± 0.02	8 ± 0.01	–	–	–	7 ± 0.02	7 ± 0.03	–	8 ± 0.07	7 ± 0.02	7 ± 0.02	9 ± 0.05	–	8 ± 0.05
Bacillus pumilus 11778	8 ± 0.25	7 ± 0.01	8 ± 0.05	–	8 ± 0.01	–	–	9 ± 0.01	7 ± 0.02	–	–	–	–	–	7 ± 0.02	–	–
Pseudomonas aeruginosa 71	–	–	–	–	–	8 ± 0.03	–	–	–	–	–	–	–	–	–	–	–
Vibrio cholerae 759	–	8 ± 0.1	7 ± 0.02	–	9 ± 0.02	–	–	–	–	8 ± 0.2	8 ± 0.02	7 ± 0.05	–	–	–	–	–
Klebsiella pneumoniae JII14	7 ± 0.05	–	7 ± 0.02	8 ± 0.02	6 ± 0.5	–	–	–	7 ± 0.02	7.5 ± 0.03	–	7 ± 0.05	–	–	–	–	–

[a] '–' shows no measurable zone of inhibition

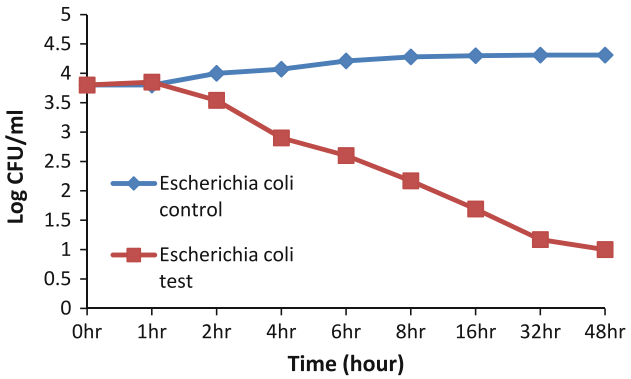


Fig. 5 Time-dependent in vitro growth curve of *E. coli 319* at their MIC values against test sample ***3b***

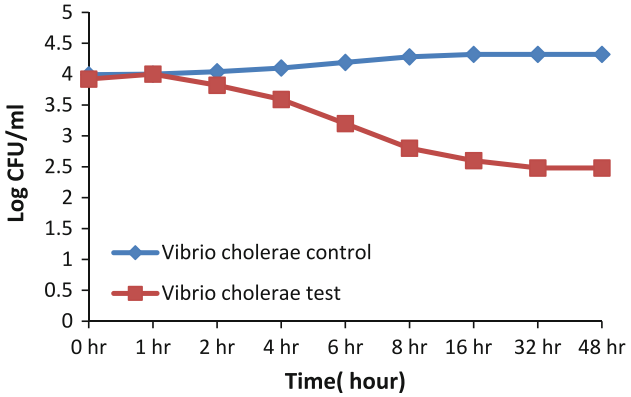


Fig. 6 Time-dependent in vitro growth curve of *Vibrio cholerae* 759 at their MIC values against test sample ***3k***

ninhydrin using 5 mol % of molecular iodine in acetonitrile at room temperature. The novelty of this versatile methodology lies in the operational simplicity, environment friendly reaction conditions, and the use of cheap and readily available starting materials.

Some of the products were identified to possess potential antimicrobial activity, especially the efficacy of the compounds against *Vibrio choleare 759* and *Shigella dysenteriae 15* demand further study as both the diseases are prevalent in our geographic region.

Experimental section

Chemistry

General methods

Melting points were determined with a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a JASCO FTIR (model 410) in KBr pellets. ESI-MS (positive) was conducted using LC-ESI-Q-TOF Micromass spectrometer. ^{1}H and ^{13}C NMR spectra were

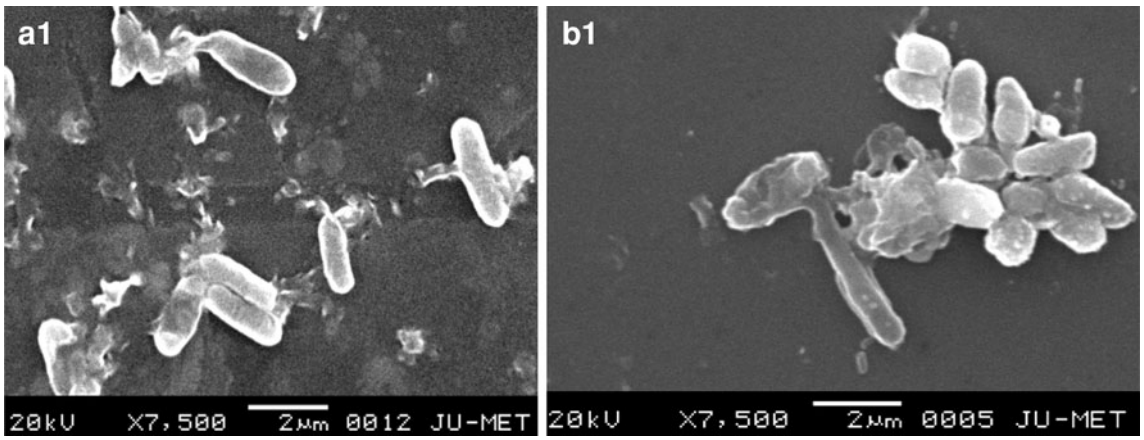


Fig. 7 SEM micrographs of microbes: ($\mathbf{a}_1$) before and ($\mathbf{b}_1$) after treatment with corresponding MIC concentration of test sample **3b** against *E. Coli 319*

Table 6 Antifungal activities of chemically synthetic compound against different fungi strains

Sample drugs[a]	Name of the of organism and minimum inhibitory concentration (μg/ml) of test			
	Aspergillus Niger	*Candida albicans*	*Candida Nigertropicalis*	*Cryptococcus neoformans*
3a	1,000	1,000	1,000	–
3b	1,000	1,000	2,000	–
3c	–	–	–	–
3d	–	–	–	–
3e	1,000	2,500	2,000	1,500
3f	–	–	–	–
3g	–	–	–	–
3h	–	–	–	–
3i	–	–	–	–
3j	–	–	–	–
3k	–	–	–	–
3l	1,000	–	1,000	–
3m	–	–	–	–
3n	–	–	–	–
3o	–	–	–	–
3p	–	–	–	–
3q	–	–	–	–
Fluconazole	10	4	8	8

[a] With respect to Fluconazole; a standard antifungal antibiotic

Table 7 Zone of inhibition of the tested compounds

Sample no.	Name of the of organism and the diameter of inhibition zones in mm			
	Aspergillus Niger	*Candida albicans*	*Candida Nigertropicalis*	*Cryptococcus neoformans*
3a	10 ± 0.18	9.0 ± 0.23	8.2 ± 0.15	–
3b	7.6 ± 0.4	10 ± 0.15	8.0 ± 0.05	–
3c	–		–	
3d	–		–	
3e	12 ± 0.17	10 ± 0.18	8..0 ± 0.12	8 ± 0.15
3f	–		–	
3g	–		–	
3h	–		–	
3i	–		–	
3j	–		–	
3k	–		–	
3l	8.0 ± 0.10	–		8.0 ± 0.20
3m	–		–	
3n	–		–	
3o	–		–	
3p	–		–	
3q	–		–	

taken on a Bruker 300 MHz DPX spectrometer at 300 and 74.99 MHz, respectively, with tetramethylsilane as internal standard and the chemical shifts are reported in δ units. Ninhydrin, molecular iodine, and aniline derivatives were purchased from Aldrich Chemical Ltd (USA). Organic solvents used for the chemical synthesis and for chromatography were acquired from E. Merck (India) and were of analytical grade. All chromatographic purifications were performed with neutral alumina obtained from SRL (India). Thin-layer chromatography was performed on pre-coated silica gel 60 F254 aluminum sheets (E. Merck, Germany) using 30 % ethyl acetate-hexane as solvent and the spots were developed using iodine or Liebermann-Burchard reagent.

*General method of preparation of 2-mono/2, 2-bis-(4-amino-phenyl)-indan-1,3-diones (3**a–q**)*

Appropriate amount (1.68 mmol) of ninhydrin (**1**) was dissolved in 15 ml of acetonitrile in a 50 ml RB flask,

catalytic amount (5 mol %) of molecular iodine was added, and the mixture was stirred at room temperature for about 10 min. Then the aniline derivative **2a** (3.36 mmol) was added to the solution and the reaction mixture was stirred at ambient temperature for 2 h. After completion of the reaction (checked by TLC), the contents of the reaction mixture were evaporated to dryness. The residue was digested with ethyl acetate and poured into a separating funnel, and then 30 ml of 10 % aqueous $Na_2S_2O_4$ solution was added. The organic layer was separated followed by extraction of the aqueous layer once with 25 ml of ethyl acetate. All the organic layers were mixed together, washed thoroughly with water, dried over sodium sulfate, and evaporated to dryness in a rotary evaporator under reduced pressure. The crude product was chromatographed over neutral alumina, which on eluting with a mixture of petroleum ether-ethyl acetate in different ratios yielded the respective products.

Experimental protocol in aqueous solution In a 50 ml RB flask, 1.68 mmol of Ninhydrin was dissolved in 10 ml distilled water, a pinch of iodine and potassium iodide were added subsequently followed by the addition of 2-Methoxyaniline (3.36 mmol). The reaction mixture was stirred at ambient temperature and within 15 min, the product precipitates out of the solution. The content of the reaction mixture was filtered, washed with water, and dried under vacuum. The residue was further recrystallized from chloroform.

Protocol for cross-over experiment Cross-over experiment was performed with 2.0 mmol of ninhydrine dissolved in a 50 ml RB flask containing 20 ml acetonitrile. Catalytic amount of molecular iodine (5 mol%) was added to the mixture and stirred for 10 min at room temperature. A mixture of 2-methoxyaniline and 2-bromoaniline one equivalent each was added and stirred for 2 h. After completion of the reaction (checked by TLC), the contents of the reaction mixture were evaporated to dryness. The residue was then purified as stated earlier.

*2,2-Bis-(4-amino-phenyl)-indan-1,3-dione (**3a**)*

Brown solid, Mp 215–217 °C; R_f (30 % Ethyl acetate-hexane) 0.65; IR (KBr, cm^{-1}) ν 3472, 3376, 1698, 1619, 1511, 1258, 814; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.14 (4H, s), 6.48 (4H, d, $J = 8.4$ Hz), 6.75 (4H, d, $J = 8.4$ Hz), 8.04 (4H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 66.3 (C), 113.8 (4xCH), 123.8 (2xCH), 125.0 (2xC), 129.2 (4xCH), 136.9 (2xCH), 140.9 (2xC), 148.1 (2xC), 200.7 (2xC). HRMS [ESI]: *m/z* calcd for $C_{21}H_{16}N_2O_2Na$: $[M + Na]^+$ 351.1109; found: 351.1085.

*2,2-Bis-(4-amino-3-methyl-phenyl)-indan-1,3-dione (**3b**)*

Brown solid, Mp 230–232 °C; R_f (30 % Ethyl acetate-hexane) 0.70; IR (KBr, cm^{-1}) ν 3462, 3378, 1707, 1626, 1505, 1257, 810; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.07 (6H, s), 3.59 (4H, s), 6.58 (2H, d, $J = 8.4$ Hz), 6.90–6.93 (4H, m), 7.83–7.86 (2H, m), 8.03–8.06 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 17.6 (2xCH_3), 66.3 (C), 113.7 (2xCH), 120.9 (2xC), 123.7 (2xCH), 125.3 (2xC), 126.8 (2xCH), 129.9 (2xCH), 136.7 (2xCH), 140.9 (2xC), 146.0 (2xC) 200.6 (2xC). HRMS [ESI], *m/z* calcd for $C_{23}H_{20}N_2O_2Na$: $[M + Na]^+$ 379.1422; found: 379.1426.

*2,2-Bis-(4-amino-3-methoxy-phenyl)-indan-1,3-dione (**3c**)*

Brown solid, Mp 198–200 °C; R_f (30 % Ethyl acetate-hexane) 0.70; IR (KBr, cm^{-1}) ν 3430, 3346, 1702, 1618, 1498, 1254, 813; ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.73 (6H, s), 3.79 (4H, s), 6.58–6.66 (4H, m), 6.72 (2H, s), 7.84–7.87 (2H, m), 8.04–8.06 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.2 (2x-OCH_3), 66.6 (C), 110.9 (2xCH), 113.2 (2xCH), 121.3 (2xCH), 123.8 (2xCH), 125.3 (2xC), 136.7 (2xCH), 137.4 (2xC), 140.9 (2xC), 146.1 (2xC) 200.2 (2xC). HRMS [ESI], *m/z* calcd for $C_{23}H_{20}N_2O_4Na$: $[M + Na]^+$ 411.1321; found: 411.1320.

*2,2-Bis-(4-amino-3-chloro-phenyl)-indan-1,3-dione (**3d**)*

Brown solid, Mp 220–222 °C; R_f (30 % Ethyl acetate-hexane) 0.70; IR (KBr, cm^{-1}) ν 3235, 2945, 1704, 1456, 1324, 1117, 776; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.51 (4H, s), 6.72–6.80 (4H, m), 6.86 (2H, s), 8.07 (4H, s); ^{13} C NMR (75 MHz, DMSO-d_6) δ 65.0 (C), 115.3 (2xCH), 116.8 (2xC), 124.1 (2xCH), 125.6 (2xC), 127.8 (2xCH), 128.7 (2xCH), 137.1 (2xCH), 140.6 (2xC), 144.4 (2xC), 199.5 (2xC). HRMS [ESI], *m/z* calcd for $C_{21}H_{14}Cl_2N_2O_2Na$: $[M + Na]^+$ 419.0330; found: 419.0365.

*2,2-Bis-(4-amino-3-bromo-phenyl)-indan-1,3-dione (**3e**)*

Brown solid, Mp 190–192 °C; R_f (30 % Ethyl acetate-hexane) 0.75; IR (KBr, cm^{-1}) ν 3457, 3362, 1696, 1618, 1491, 1261, 807,771; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (4H, s), 6.80 (2H, d $J = 8.4$ Hz), 7.02 (2H, dd $J = 8.4$, 1.8 Hz), 7.28 (2H, d $J = 1.8$ Hz), 7.88–7.90 (2H, m), 8.05–8.08 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 64.8 (C), 107.1 (2xC), 115.2 (2xCH), 124.1 (2xCH), 126.1 (2xC), 128.4 (2xCH), 131.7 (2xCH), 137.1 (2xCH), 140.6 (2xC), 145.5 (2xC), 199.4 (2xC). HRMS [ESI], *m/z* calcd for $C_{21}H_{14}Br_2N_2O_2Na$: $[M + Na]^+$ 506.9320; found: 506.9310.

*2,2-Bis-(4-amino-3-iodo-phenyl)-indan-1,3-dione (**3f**)*

Brown solid, Mp 180–182 °C; R_f (30 % Ethyl acetate-hexane) 0.78; IR (KBr, cm^{-1}) ν 3488, 3370, 1699, 1632, 1510, 1341, 1258, 774; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.12 (4H, s), 6.66 (2H, d $J = 8.4$ Hz), 7.04 (2H, dd $J = 8.4$, 2.1 Hz), 7.47 (2H, d $J = 2.1$ Hz), 7.88–7.90 (2H, m), 8.05–8.07 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 64.5 (C), 82.8 (2xC), 114.1 (2xCH), 124.0 (2xCH), 126.7 (2xC), 129.2 (2xCH), 137.1 (2xCH), 137.9 (2xCH), 140.5 (2xC), 148.2 (2xC), 199.5 (2xC). HRMS [ESI], *m/z* calcd for $C_{21}H_{14}I_2N_2O_2Na$: $[M + Na]^+$ 602.9042; found: 602.9019.

*2,2-Bis-(4-amino-3-nitro-phenyl)-indan-1,3-dione (**3g**)*

Brown solid, Mp 198–200 °C; R_f (30 % Ethyl acetate-hexane) 0.65; IR (KBr, cm^{-1}) ν 3459, 3370, 1701, 1619, 1499, 1255, 812; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.03 (2H, d $J = 9.0$ Hz), 7.21–7.25 (2H, m), 7.71 (4H, s), 7.71 (2H, s), 8.08–8.17 (4H, m); ^{13} C NMR (75 MHz, DMSO-d_6) δ 64.3 (C), 120.4 (2xCH), 123.8 (2xC), 124.3 (2xCH), 124.8 (2xCH), 129.5 (2xC), 135.6 (2xCH), 137.5 (2xCH), 140.5 (2xC), 145.8 (2xC), 198.8 (2xC). HRMS [ESI], *m/z* calcd for $C_{21}H_{14}N_4O_6Na$: $[M + Na]^+$ 441.0811; found: 441.0810.

*2,2-Bis-(4-amino-3,5-dimethyl-phenyl)-indan-1,3-dione (**3h**)*

Brown solid, Mp 224–226 °C; R_f (30 % Ethyl acetate-hexane) 0.70; IR (KBr, cm^{-1}) ν 3496, 3400, 1703, 1631, 1508, 1244, 1051, 850, 764; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.09 (12H, s), 3.56 (4H, s), 6.81 (4H, s), 7.83–7.85 (2H, m), 8.03–8.06 (2H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.1 (4xCH_3), 66.4 (C), 120.5 (4xC), 123.7 (2xCH), 125.0 (2xC), 127.9 (4xCH), 136.7 (2xCH), 140.9 (2xC), 143.8 (2xC), 200.6 (2xC). HRMS [ESI], *m/z* calcd for $C_{25}H_{24}N_2O_2Na$: $[M + Na]^+$ 407.1735; found: 407.1754.

*2,2-Bis-(4-amino-2-fluoro-5-methyl-phenyl)-indan-1,3-dione (**3i**)*

Brown solid, Mp 206–208 °C; R_f (30 % Ethyl acetate-hexane) 0.75; IR (KBr, cm^{-1}) ν 3474, 3393, 1706, 1627, 1487, 1240, 779; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.04 (6H, s), 3.71 (4H, s), 6.31 (2H, d $J = 12.6$ Hz), 6.83 (2H, d $J = 8.1$ Hz), 7.81–7.84 (2H, m), 8.02–8.05 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 16.9 (2xCH_3), 62.7 (C), 100.0 (CH), 100.4 (CH), 109.0 (C), 109.2 (C), 117.0 (2xC), 123.7 (2xCH), 131.6 (CH), 131.7 (CH), 136.4 (2xCH), 140.1(2xC), 148.4 (C), 148.5 (C), 157.5 (C), 160.7 (C), 197.9 (2xC). HRMS [ESI], *m/z* calcd for $C_{23}H_{18}F_2N_2O_2Na$: $[M + Na]^+$ 415.1234; found: 415.1227.

*2,2-Bis-(4-methylamino-phenyl)-indan-1,3-dione (**3j**)*

Brown solid, Mp 195–197 °C; R_f (30 % Ethyl acetate-hexane) 0.72; IR (KBr, cm^{-1}) ν 3406, 1700, 1610, 1519, 1325, 1256, 816, 528; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.79 (6H, s), 3.71 (2H, bs), 6.50–6.53 (4H, m), 7.06–7.09 (4H, m) 7.83–7.87 (2H, m), 8.03–8.07 (2H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.1 (2xCH_3), 66.3 (C), 111.9 (4xCH), 123.5 (2xCH), 126.2 (2xC), 129.3 (4xCH), 135.7 (2xCH), 141.2 (2xC), 148.4 (2xC), 200.8 (2xC). HRMS [ESI], *m/z* calcd for $C_{23}H_{20}N_2O_2Na$: $[M + Na]^+$ 379.1422; found: 379.1443.

*2-(4-Amino-2-chloro-5-methoxy-phenyl)-2-(5-chloro-2-methoxy-phenylamino)-indan-1,3-dione (**3k**)*

Brown solid, Mp 210–212 °C; R_f (30 % Ethyl acetate-hexane) 0.80; IR (KBr, cm^{-1}) ν 3479, 3385, 1744, 1709, 1593, 1509, 1247, 1173, 959, 742; ^{1}H NMR (600 MHz, DMSO-d_6) δ 3.49 (3H, s), 3.70 (3H, s), 5.27 (2H, s), 5.77 (1H, s), 6.60 (1H, s), 6.69 (1H, dd $J = 2.4$, 9.0 Hz), 6.78 (1H, s), 6.83 (1H d $J = 8.4$ Hz), 6.87 (1H, d $J = 2.4$ Hz), 8.01–8.05 (4H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 55.4 (CH_3), 55.8 (CH_3), 69.1 (C), 111.3 (CH), 113.3 (CH), 113.8 (CH), 115.3 (CH), 117.7 (CH), 117.9 (C), 122.5 (C), 123.5 (C), 124.3 (2xCH), 134.8 (C), 136.7 (2xCH), 139.9 (3xC), 144.8 (C), 146.1 (C) 195.1 (2xC). HRMS [ESI], *m/z* calcd for $C_{23}H_{18}Cl_2N_2O_4Na$: $[M + Na]^+$ 479.0541; found: 479.0540.

*2-(2-Amino-3,5-dichloro-phenyl)-2-(2,4-dichloro-phenylamino)-indan-1,3-dione (**3l**)*

Brown solid, Mp 216–218 °C; R_f (30 % Ethyl acetate-hexane) 0.78; IR (KBr, cm^{-1}) ν 3478, 3380, 1709, 1616, 1589, 1497, 1246, 948, 742; ^{1}H NMR (300 MHz, DMSO-d_6) δ 5.93 (2H, s), 6.31 (1H, s), 6.80 (2H, s), 6.98 (1H, s), 7.18 (1H, s), 7.32 (1H, d $J = 8.4$ Hz), 8.06 (4H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 68.8 (C), 115.6 (CH), 115.9 (C), 116.9 (CH), 118.5 (C), 119.0 (C), 119.3 (CH), 124.4 (C), 124.4 (CH), 129.7 (C), 130.4 (CH), 130.9 (C), 131.2 (CH), 136.9 (2xCH), 139.9 (2xC), 141.4 (C), 146.4 (C), 194.4 (2xC). HRMS [ESI], *m/z* calcd for $C_{21}H_{12}Cl_4N_2O_2Na$: $[M + Na]^+$ 486.9551; found: 486.9552.

*2-(2-Amino-4,5-dimethyl-phenyl)-2-(3,4-dimethyl-phenylamino)-indan-1,3-dione (**3m**)*

Yellow solid, Mp 225–227 °C; R_f (30 % Ethyl acetate-hexane) 0.80; IR (KBr, cm^{-1}) ν 3368, 3336, 1712, 1614, 1502, 1288, 1046, 777; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.05 (3H, s), 2.09–2.10 (9H, s), 4.04 (1H, s), 4.54 (1H, s), 5.07

(1H, s), 6.33–6.37 (1H, m), 6.43 (1H, s), 6.54 (1H, d $J = 1.5$ Hz), 6.80 (1H, d $J = 8.1$ Hz), 7.02 (1H, s), 7.45–7.50 (1H, m), 7.75–7.80 (2H, m), 7.89 (1H, d $J = 7.5$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ 18.3 (CH_3), 19.0 (CH_3), 19.7 (CH_3), 19.9 (CH_3), 74.6 (C), 93.3 (C), 109.7 (CH), 112.3 (CH), 116.7 (CH), 121.3 (CH), 122.3 (CH), 123.4 (C), 125.2 (CH), 125.8 (CH), 128.9 (CH), 129.3 (CH), 133.8 (C), 135.0 (C), 136.2 (CH), 138.5 (C), 144.7 (C), 145.8 (C), 152.8 (C), 200.5 (2xC). HRMS [ESI], *m/z* calcd for $C_{25}H_{24}N_2O_2Na$: $[M + Na]^+$ 407.1735; found: 407.1746.

*2-(4-Dimethylamino-phenyl)-2-hydroxy-indan-1,3-dione (**3n**)*

Brown solid, Mp 200–202 °C; R_f (30 % Ethyl acetate-hexane) 0.80; IR (KBr, cm^{-1}) ν 3469, 1745, 1610, 1529, 1367, 1180, 979, 801; ^{1}H NMR (300 MHz, $CDCl_3$) 2.91 (6H,s), 3.17–3.18 (1H, m), 6.62 (2H, d $J = 8.7$ Hz), 7.31 (2H, d $J = 9.0$ Hz), 7.86–7.89 (2H, m), 8.01–8.04 (2H, m); ^{13}C NMR (75 MHz, DMSO-d_6) δ 39.8 (2xCH$_3$), 79.1 (C), 112.0 (2xCH), 123.6 (2xCH), 124.0 (C), 127.0 (2xCH), 136.9 (2xCH), 140.2 (2xC), 150.1 (C), 199.9 (2xC). HRMS [ESI], *m/z* calcd for $C_{17}H_{15}NO_3Na$: $[M + Na]^+$ 304.9050; found: 304.9010.

*2-(4-Diethylamino-phenyl)-2-hydroxy-indan-1,3-dione (**3o**)*

Brown solid, Mp 195–197 °C; R_f (30 % Ethyl acetate-hexane) 0.78; IR (KBr, cm^{-1}) ν 3452, 2972, 1747, 1708, 1603, 1523, 1349, 1197, 1135, 783, 733; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.00–1.03 (6H, m), 3.25–3.29 (4H, m), 6.55–6.60 (2H, m), 7.01 (2H, d, $J = 7.8$ Hz), 8.03 (4H, s); ^{13} C NMR (75 MHz, DMSO-d_6) δ 12.4 (2xCH$_3$), 43.6 (2xCH$_2$), 79.2 (C), 111.2 (2xCH), 122.7 (C), 123.6 (2xCH), 127.5 (2xCH), 137.0 (2xCH), 140.2 (2xC), 147.2 (C), 200.8 (2xC). HRMS [ESI], *m/z* calcd for $C_{19}H_{19}NO_3Na$: $[M + Na]^+$ 332.1263; found: 332.1243. [The –OH signal is incorporated within the solvent pick]

*2-(4-Amino-2-methoxy-phenyl)-2-hydroxy-indan-1,3-dione (**3p**)*

Yellow solid, Mp 210–212 °C; R_f (30 % Ethyl acetate-hexane) 0.80; IR (KBr, cm^{-1}) ν 3391, 3324, 1709, 1608, 1248, 1027, 850, 727; ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.11 (3H, s), 5.18 (2H, s), 6.04 (1H, s), 6.23 (1H, d $J = 8.1$ Hz), 6.08 (1H, s), 7.30 (1H, d $J = 8.1$ Hz), 7.99 (4H, s); ^{13}C NMR (75 MHz, DMSO-d_6) δ 54.7 (CH_3), 75.5 (C), 97.0 (CH), 106.0 (CH), 113.6 (C), 123.1 (2xCH), 128.4 (CH), 136.2 (2xCH), 139.9 (2xC), 150.2 (C), 155.6 (C), 200.7 (2xC). HRMS [ESI], *m/z* calcd for $C_{16}H_{13}NO_4Na$: $[M + Na]^+$ 306.0742; found: 306.0723.

*2-(5-Amino-quinolin-8-yl)-2-hydroxy-indan-1,3-dione (**3q**)*

Brown solid, Mp 201–203 °C; R_f (30 % Ethyl acetate-hexane) 0.62; IR (KBr, cm^{-1}) ν 3374, 3071, 2787, 1739, 1707, 1589, 1472, 1232, 855, 714; ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.08 (2H, s), 6.80 (1H, d $J = 7.8$ Hz), 7.06 (1H, s), 7.17–7.21 (1H, m), 7.84 (1H, d $J = 7.8$ Hz), 8.00 (4H, bs), 8.08 (1H, s), 8.48 (1H, d $J = 8.4$ Hz); ^{13}C NMR (75 MHz, DMSO-d_6) δ, 77.1 (C), 107.1 (CH), 117.4 (C), 119.2 (CH), 123.4 (C), 123.6 (2xCH), 130.0 (CH), 131.6 (CH), 136.0 (2xCH), 140.8 (C), 144.7 (C), 145.6 (2xC), 148.4 (CH), 200.2 (2xC). HRMS [ESI], *m/z* calcd for $C_{18}H_{12}N_2O_3Na$: $[M + Na]^+$ 327.0746; found: 327.07424.

*Crystal data for **3a***

Brown rectangular shaped crystals were grown from chloroform-hexane. $C_{21}H_{16}N_2O_2$, Mr = 328.36. Space group monoclinic C2/c. Lattice constants (Å): $a = 12.7349(9)$, $b = 17.3728(13)$, $c = 14.8084(11)$, $\alpha = 90$, $\beta = 93.443(3)$, $\gamma = 90$, cell volume $V = 3270.3(4)$ Å^3, formula units/cell $Z = 8$, number of independent reflections 2878, after convergence R1 = 0.0383, wR2 = 0.1499.

*Crystal data for **3k***

Brown rectangular shaped crystals were grown from chloroform-hexane. $C_{23}H_{18}Cl_2N_2O_4$, Mr = 457.29. Space group triclinic P-1. Lattice constants (Å): $a = 8.9748(9)$, $b = 8.9748(9)$, $c = 8.9748(9)$, $\alpha = 8.9748(9)$, $\beta = 99.469(4)$, $\gamma = 99.469(4)$, cell volume $V = 1061.54(18)$ Å^3, formula units/cell $Z = 2$, Number of independent reflections 9539, after convergence R1 = 0.0519, wR2 = 0.1967.

Pharmacological studies

Materials and methods

Microorganisms The microorganisms used in this study consisted of eight strains of bacteria: *Staphylococcus aureus* 29737, *Shigella dysenteriae* 15, *Micrococcus luteus* ATCC 9341, *Escherichia coli* 319, *Bacillus pumilus 11778*, *Pseudomonas aeruginosa* 71, *Vibrio cholerae* 759, and *Klebsiella pneumoniae J/I/4*, and four fungi: *Aspergillus niger*, *Candida albicans*, *C. tropicalis*, and *Cryptococcus neoformans.* All the strains were clinical isolates from human beings. The strains were identified using Barrow and Feltham's method (Barrow and Feltham, 1993). These were obtained from the

Division of Microbiology, Dept. of Pharmaceutical Technology, Jadavpur University, Kolkata-700 032, India. The bacterial strains were grown in Mueller–Hinton Agar (Merck India Ltd.) at 37 °C for 24 h, while the fungi were grown on Sabouraud dextrose agar at 28 °C for 3–5 days.

Preparation of inoculums The desired bacteria and fungi were cultured in Mueller–Hinton Broth (MHB) at 37 °C for 24 h and in Sabouraud dextrose broth (SDB) at 28 °C for 72 h, respectively. The microorganism suspension was prepared by matching a 0.5 McFarland standard (McFarland, 1907).

Preparation of stock solution All the test samples, screened for their antimicrobial activity, were dissolved up to 4 % DMSO to get the concentration of 1 mg/ml and were used as stock solution.

Determination of minimum inhibitory concentration (MIC) The antimicrobial activities of the test samples were evaluated by finding the minimum inhibitory concentration (MIC) by agar dilution and broth dilution methods (Chattopadhyay *et al*., 2002). For broth dilution assay, 0.1 ml standardized suspensions of bacteria (2×10^6 cfu/ml) were added to Mueller–Hinton broth containing test sample at the concentration 1–1,000 µg/ml with appropriate antibiotic control and incubated at 37 °C overnight for bacteria. For agar dilution assay, previously prepared dilutions of the test sample, with appropriate antibiotic control, were prepared in Mueller–Hinton Agar or Sabouraud dextrose agar. The mixtures were added into sterile petri-dishes after complete mixing. A loop of each standardized suspension (2×10^6 cfu/spot for bacteria and 2×10^5 spores/spot for fungi) of microorganism was inoculated on prepared agar plates by drawing a stripe. The inoculated plates were then incubated at 37 °C for 24 h for bacteria and at 28 °C for 96 h for fungi. The experiments were done in triplicate. The lowest concentration required to inhibit the growth of microorganism, i.e., the concentration at which no microorganism colony or less than 5 colonies were visible within 19–38 h, was considered as the MIC (Wang *et al*., 2006).

Antimicrobial assay (evaluation of zone of inhibition) Qualitative antimicrobial property of test samples was evaluated by disk diffusion method, as per NCCLS protocol (NCCLS 1993). For sensitivity testing, 0.1 ml of 2×10^6 cfu/ml of bacterial suspension or 0.1 ml of 2×10^5 spores/ml of fungal spore suspension was transferred to a freshly prepared Mueller–Hinton Agar plate or Sabouraud dextrose plate. A sterile bent glass rod was used to spread the suspension to achieve uniform growth. Then, sterile paper disks (5 mm diameter) impregnated with different sample concentrations were placed aseptically on sensitivity plates. The plates were then incubated at 37 °C overnight for bacteria and 28 °C for 96 h for fungi. The sensitivity was recorded by measuring the clear zone of inhibition on agar surface around the disks.

Determination of the killing rate The quantitative antimicrobial activity of the synthesized product was measured by viable cell count experiments (Rhim *et al*., 2009). The microorganism suspension was diluted by sterile distilled water to 2×10^6 cfu/ml. The standardized suspension (1 ml) of bacteria was added into Mueller–Hinton broth containing test sample (**3b**, **3k**) at their MIC ranges, while another test tube without drug sample served as control. The mixtures were then incubated at 37 °C with shaking on a platform shaker at 200 rpm. Aliquots (1 ml each) of sample were withdrawn at 0, 1, 2, 4, 6, 8 h till 48 h, spread on nutrient agar plates, and incubated at 37 °C overnight. The numbers of colonies were counted and reduction in plate colonies was calculated by comparing with control plates.

Scanning electron microscopic (SEM) study Scanning electron microscopic observations were carried out on bacterial cells after 12 h incubation in Mueller–Hinton broth at 37 °C. The suspension was divided into two portions and test sample (**3b**) was added to one portion so as to achieve a concentration at MIC range. The other portion was left untreated as a control. After 24 h incubation, the cells from both tubes were harvested by centrifugation, washed twice, and fixed in 3 % (v/v) glutaraldehyde buffered with 0.1 M sodium phosphate buffer (pH 7.2) for an hour at room temperature. They were dehydrated in a graded alcohol series. The specimens were dried and mounted onto stubs using double-sided carbon tape, and then coated with a thin layer of gold using a microscope sputter coater for 1 min at 20 mA. The coated sample was then examined in a JEOL JSM 6360 Scanning Electron Microscope (Hayat, 1981).

Supplementary data

^{1}H and ^{13}CNMR spectra of all new compounds associated with this article can be found in the online version. Crystallographic data in CIF format available free of charge via the Internet at CCDC 816861-816863 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2

1EZ, UK; fax: +44-1223-336033; or deposit@ccdc.cam.ac.uk).

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