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Research Article

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Synthesis and biological activity studies of novel heterocyclic compounds

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ABSTRACT

We herein report the synthesis of the novel heterocyclic compounds ***(1),(2)&(3),*** *by the Grignard addition of 2 thiophenyl magnesium bromide to phenanthrene-1,2- dione, benzil and acenaphthene-1,2-dione respectively The synthesized novel heterocyclic compounds were screened for antibacterial, antifungal and cytotoxic activity studies.*

Keywords: Grignard addition, 2-thiophenyl magnesium bromide, antibacterial activity, antifungal activity, cytotoxic activity

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INTRODUCTION

Sulphur and nitrogen containing heterocyclic compounds in general are shown to poses various biological activities [1]. In view of our interest in heterocyclic synthesis [2,3], we are reporting the synthesis of novel heterocyclic compounds namely, 9,10-dihydro-9,10-di-(thiophen-2-yl)phenanthrene-9,10-diol **(1)**, 1,2-diphenyl-1,2-di(thiophen-2-yl)ethane-1,2-diol **(2)** and **(3)** and evaluation of their biological activity

EXPERIMENTAL SECTION

Materials and Methods

All m.ps. are uncorrected. The purity of the compounds was checked by Thin Layer Chromatography (TLC) on silica gel. ^{1}H NMR spectra were recorded in deutero chloroform ($CDCl_3$) using tetra methyl silane (TMS) as an internal standard on a BRUKER 300 spectrometer at 400 mega Hertz (MHz). ^{13}C NMR were recorded on a BRUKER 300 spectrometer at 100 MHz and mass spectra on JEOL DX – 303 spectrometer. Tetrahydrofuran was freshly distilled from sodium benzophenone ketyl before use. The reactions were carried out in a Schlenk type glass apparatus under nitrogen atmosphere

General procedure for the synthesis of compounds (1), (2) and (3)

To a solution of 2-thiophenyl magnesium bromide in dry THF at 0°C under N_2 atmosphere [prepared from magnesium (Mg)(0.03mol) and2-bromo thiophene (0.03 mol)], the dione (0.01mol) in dry THF was added dropwise over a period of 15 – 20 minutes. After completion of addition, the mixture was stirred at 0° C under N_2 atmosphere for 2 h followed by stirring at room temperature under N_2 atmosphere for 3h. After the completion of reaction as evidenced by TLC, the reaction was quenched with a saturated solution of ammonium chloride (NH_4Cl) (50mL). The product was extracted with diethyl ether (2x50ml). The ether layer was washed with water followed by brine and dried over anhydrous sodium sulphate (Na_2SO_4) and evaporated. Purification of the crude product by column chromatography on silica gel (100 – 200 mesh) using hexane – ethyl acetate (9:1) as eluent gave the corresponding product.

Antimicrobial activity

Antibacterial activity:

The antibacterial activity studies of the compounds were screened by using cup-plate diffusion method. The bacterial organisms used included both gram positive and gram negative strains like *S.aureus, E. faecalis, S. typi, V.*

chlolerae and *K. pneumonia*. Sensitivity plates were seeded with a bacterial inoculums of 1x 10^6 CIU/ml and each well diameter(10mm) was loaded with 0.1 ml of test compound solution (100µg/ml) in DMF, so that concentration of each test compounds was 10µg/ml. The zones of inhibition were recorded after incubation for 24 h using vernier caliper. The standard drug used is streptomycin.

Antifungal activity :
The organic compounds **(1),(2) &(3)** were screened for their antifungal activity against the pathogenic fungi *Aspergillus fumicaytus, Fusarium spp, Trichophyton mentagrothyte, Trichophyton rubrum and Penicillium spp.* For antifungal study, the results were taken for consequent two days from 1st day of incubation and given importance up to 2nd day of incubation. The standard used as reference is Amphotericin-B.

Antimicrobial assay
Screening of antimicrobial activity was carried out in the following sequence
(i) Preparation of nutrient agar (ii) Preparation of Mc Ferland standards (iii) Inoculums preparation. (iv) *In vitro* Antimicrobial Sensitivity Determination by Agar well diffusion method.

(i) Preparation of nutrient agar
The nutrient agar was prepared by dissolving beef extract(1.5g), peptone(0.5g), yeast extract (1.5g), sodium chloride (0.5g) and agar (1.5g) 100ml of distilled water. The pH was adjusted to 7.2 followed by sterilization in an autoclave at 121^{0}C / 15LB for 15 minutes. The sterile molten agar media was then cooled to 50^{0}C. About 15 ml of the media was poured on a sterile petri-plate and allowed to cool to room temperature.

(ii) Preparation of 0.5 Mc Ferland standards
0.5ml of solution A (1.175g of barium chloride in 100 ml of distilled water) was added to 99.5 ml of solution B (1 ml of 0.36 N sulphuric acid in 100 ml of distilled water) and mixed well with magnetic stirrer, then distributed in test tubes with a screw cap of the same size as those containing the bacterial/ fungal culture.

(iii) Preparation of bacterial and fungal inoculums.
The cooled sterile broth medium was poured into sterile petri-plates having a uniform depth of 4mm; this is equivalent to approximately 25 ml in a 90mm plate. Once the medium had solidified then the culture was inoculated on the medium. The turbidity of the culture was adjusted with sterile broth so as to correspond to 0.5 Mc Ferland standards. Immediately after standardisation, a sterile cotton swab was immersed in the bacterial/ fungal suspension and then rotated and compressed against the wall of the test tube, so as to remove the excess fluid.

(iv) *In vitro* Antimicrobial Sensitivity Determination by Agar well diffusion method.
In vitro antimicrobial sensitivity of the antibiotics and the test compounds synthesised were determined by well diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) [4]. The well diffusion test was performed using medium, as per the procedure described by Magaldi *et al* [5] .A sterilized 10 mm cork borer was used to make agar wells on the sterile nutrient agar plates. 24 hours sub cultured bacteria/ fungi were inoculated in the petri-plates, with a sterile cotton swab. Compounds were dissolved in DMSO solvent separately and poured in the wells with varying concentrations ranging from 50µL, 100µL, 150µL and 200µL, using a micropipette. 100% DMSO was used as a control. The plates were incubated for 24 hours at 37^{0}C. Antibiotic Streptomycin was used as a reference antibacterial agent and Amphotericin-B were used as a reference antifungal agent. The tests were carried out in triplicates.

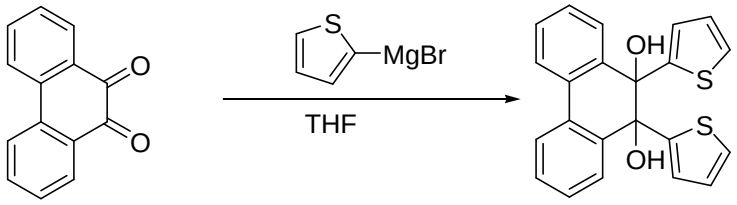
Cytotoxic activity.
In order to study the antitumor activity of a new drug, it is important to determine the cytotoxicity concentration of the drug. Cytotoxicity tests define the upper limit of the extract concentration, which is non-toxic to the cell line. The concentration non toxic to the cells is chosen for antiviral assay.

The organic compounds were dissolved in DMSO and different concentrations between 1000-1.953 µg / ml were prepared for cytotoxicity assay. The morphological changes of the cancer cells treated with different concentrations of the heterocyclic organic compounds (**1),(2) & (3),** were studied at different time intervals during incubation period.

RESULTS AND DISCUSSION

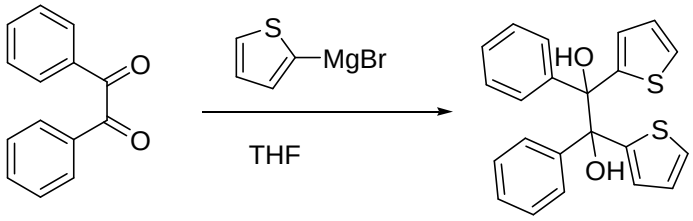
Compound **(1)** was prepared by the addition of 2-thiophenyl magnesium bromide to phenanthrene 9,10- dione at room temperature (**Scheme-I)**. The IR spectrum of (**1)** showed absence of >C=O stretching and presence of –OH stretching peak at 3496 cm^{-1} .The ^{1}HNMR spectrum of (**1)** showed D_2O exchangeable signals at 1.567 δ and 2.9 δ for -OH protons, a multiplet at 6.675 - 6.774 δ for the protons of the thiophene ring and a multiplet at 7.11-8.298 δ

for the protons of the benzene rings. The ^{13}CNMR spectrum of (**1)** showed a signal for >C – OH at 80.57δ. The mass spectrum showed m/z (M^+ + 2) peak at 378.



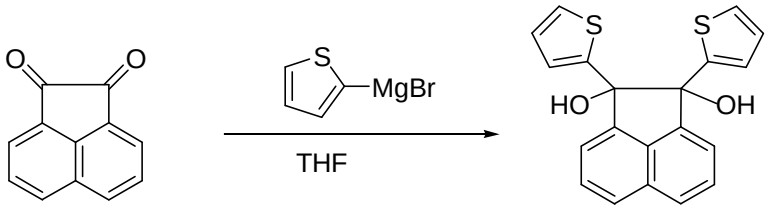
(1)

Scheme - 1



(2)

Scheme - 2



(3)

Scheme - 3

Addition of 2-thiophenyl magnesium bromide to benzil gave **(2) (Scheme - 2).** The IR spectrum of (**2**) showed the absence of >C=O Stretching and presence of -OH stretching at 3542 cm^{-1}. The ^{1}H NMR spectrum of (**2**) showed D_2O exchangeable signals at 1.5 δ and 3.3 δ for the -OH protons, a multiplet at 6.91-7.08 δ for the protons of two thiophene rings and a multiplet at 7.15-7.44 δ for the protons of the benzene rings. The ^{13}C NMR of (**2**) showed >C-OH carbon at 83.11 δ. The Mass spectrum of (**2**) showed ($M^{+\cdot}$) at 378.

The compound (**3**) was prepared by the addition of four equivalents of 2-thiophenyl magnesium bromide to acenaphthoquinone in THF at room temperature **(Scheme-3)**. The IR spectrum of **(3)** showed the absence of >C=O stretching and the presence of -OH stretching at 3524 cm^{-1}. The ^{1}H NMR spectrum of **(3)** showed D_2O exchangeable signal at 2.7 δ for the OH protons, signals in the region 6.77 to 7.40 δ for the protons of the thiophene rings, and signals in the region 7.52 to 7.92 δ for the protons of naphthalene ring. The ^{13}C NMR spectrum of (**3**) showed >C-OH carbon at 88.5 δ. The mass spectrum of (**3)** showed m/z ($M^{+\cdot}$ +2) at 352.4231.

Table I: Physical data of the Novel Heterocyclic Compounds (1-3)

Entry	Product	Molecular Formula	Melting Point (^{0}C)	Yield(%)of Product
1	**1**	$C_{22}H_{16}O_2S_2$	198 °	80
2	**2**	$C_{22}H_{18}O_2S_2$:	123-125	75
3	**3**	$C_{20}H_{14}O_2S_2$	165° C	80

Spectral data

Compound 1

IR (KBr, cm^{-1}) 3496 (O-H str.), 3100 (C-H str.), 1645 (C=C str.); **^{1}H NMR** (400 MHz, $CDCl_3$/TMS) δ (ppm): 1.567 (s, 1H, D_2O exchangeable OH proton), 2.9 (s, 1H, D_2O exchangeable OH proton), 6.675-6.774 (m, 6H, protons of thiophene ring), 7.11-8.298 (m, 8H, aromatic protons of the benzene ring); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm) : 80.57, 125.57, 126, 126.73, 128.85, 128.94, 129.1, 129.2, 131.77, 140.66, 145.02; **Mass spectrum** m/z: 378 (M^+ +2).

Compound (2):

IR (KBr, cm^{-1}) : 3542 (-OH str.), 3103, 3076 (-CH str.), 1633(C=C str.); **^{1}H NMR** (400 MHz, $CDCl_3$ / TMS) δ (ppm) : 1.5 (s, 1H, D_2O exchangeable O-H proton), 3.3 (s, 1H, D_2O exchangeable OH proton), 6.91-7.08 (m, 6H, protons of thiophene ring), 7.15-7.44 (m, 10H, due to protons of benzene ring); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm) : 83.11, 125.96, 126.39, 127.14, 127.26, 127.48, 127.75, 127.82, 141.90, 146.30; **Mass spectrum** m/z: 378 ($M^{+\cdot}$).

Compound (3):

IR (KBr, cm^{-1}) : 3524 (-OH str.), 3098 (C-H str.), 1623 (C=C str.); **^{1}H NMR** (400MHz, $CDCl_3$ / TMS) δ (ppm) : 2.7 (s, 2H, D_2O exchangeable, OH protons), 6.77 (dd, 2H, β-protons of the thiophene ring), 7.03 (dd, 2H, β-protons of the thiophene ring) 7.40 (dd, 2H, α-protons of the thiophene ring), 7.52 (d, 2H, protons of naphthalene ring) 7.67 (dd, 2H, protons of the naphthalene ring) 7.92 (d, 2H, peri protons of the naphthalene ring); **^{13}C NMR** (100 MHz, $CDCl_3$) δ (ppm) : 88.5, 121.8, 125.7, 126.52, 126.89, 126.92, 126.95, 128.87, 135.94, 144.25, 144.82; **Mass spectrum** m/z: 352.4231. (M^+ +2).

The antibacterial screening data of compounds (**1, 2 & 3**) shows very good antibacterial activity against *V. cholera and K. pneumonia.*, whereas S *S. aureus, S. typi* and *E. faecalis* showed moderate inhibition zone against these compounds. The results are presented in **Table 2.**

Table-2 – Antibacterial screening data for (1),(2) &(3).

Compound (Concentration)	Microorganisms – Zone of Inhibition in mm				
	S.aureus,	***E.faecalis***	***S. typi***	***V.chlolerae***	***K pneumonia***
(1) (10µg)	12	10	16	12	10
(2) (10µg)	10	08	12	15	11
(3)(10µg)	09	07	10	10	11
Streptomycin(10µg).	18	16	25	13	12

The results of antifungal studies are shown in **Table- 3.** It is observed that all the three organic compounds (**1,2 &3**) played significant role on pathogenic fungi *Aspergillus fumicaytus, Fusarium spp, Trichophyton rubrum and Penicillium spp.* The growth control effect was very high than the standard Amphotericin-B. The effective inhibitory activity was observed on all the two days of incubation period taken for the study. It seems from the results that sulfur in the organic compounds plays a significant role in the inhibition activity.

The cytotoxicity activity studies data are shown for compound **1, 2** and **3** in **Table-4.**

The heterocyclic compounds (**1, 2 & 3)** showed cytotoxic activity positively. Most of the cells were arrested, at the middle concentration taken, during cell and the cell nuclei became condensed and segmented after 48 h incubation which is the indication of apoptosis. The results from light microscope displayed morphological abnormality of the cells after treatment with the heterocyclic organic compounds on the contrary the untreated cells (control) did not show

these apoptotic characteristics [6]. Most of the condensed and segmented nuclei degraded after 48 h incubation. With these data, one can conclude a heterocyclic ring-dependent activity of the studied heterocyclic organic compounds on the Hep-2 cells.

Table 3. Antifungal screening data for (1), (2) & (3)

S. No	Compounds	Microorganisms	Zone of inhibition in mm after 48 h
1	**(1)**	*Aspergillus fumicatus*	10
		Fusarium spp	6
		Trichophyton mentagrophyte	5
		Trichophyton rubrum	10
		Penicillium spp	6
2	**(2)**	*Aspergillus fumicatus*	11
		Fusarium spp	6
		Trichophyton mentagrophyte	-
		Trichophyton rubrum	10
		Penicillium spp	4
3	**(3)**	*Aspergillus fumicatus*	11
		Fusarium spp	10
		Trichophyton mentagrophyte	6
		Trichophyton rubrum	10
		Penicillium spp	6
4	**Amp B**	*Aspergillus fumicatus*	6
		Fusarium spp	1
		Trichophyton mentagrophyte	5
		Trichophyton rubrum	12
		Penicillium spp	-

It is indicated that with the increase of heterocyclic organic compounds concentration, heterocyclic organic compounds (**1, 2 & 3**) induced increased cell death through apoptosis. With further increase of heterocyclic organic compound concentration, the induced cell death through apoptosis decreased dramatically as evident from the assay report. In the present study, we observed that at low to medium concentration the efficacy of the heterocyclic organic compounds was quite dependent on the specific cell type.

The result is confirmed by additional metabolic intervention experiment such as MTT assay **Fig-1, Fig-2 & Fig-3**.

Table-4-Cytotoxicity screening data for Compounds (1), (2)& (3).

S. No.	Concentration ((µg/ml)	Dilutions	Cell viability		
			Compound(1)	Compound(2)	Compound(3)
1	1000	Neat	45.78	28.15	34.17
2	500	1:1	51.23	33.90	41.77
3	250	1:2	58.90	44.78	47.78
4	125	1:4	63.15	56.78	53.78
5	62.5	1:8	69.07	67.89	59.89
6	31.25	1:16	73.44	73.44	67.88
7	15.625	1:32	78.19	78.90	73.34
8	7.8125	1:64	82.90	81.23	82.34
9	3.906	1:128	88.67	89.09	88.90
10	1.953	1:256	93.65	93.45	97.67
11	Cell control	-	100	100	100

Fig – 1- MTT Assay for compound (1)

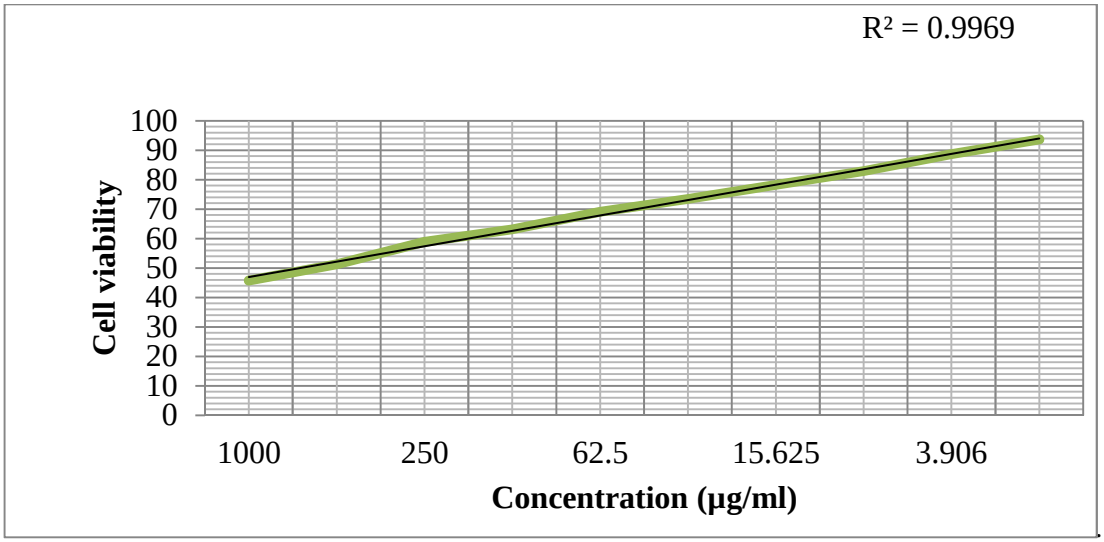
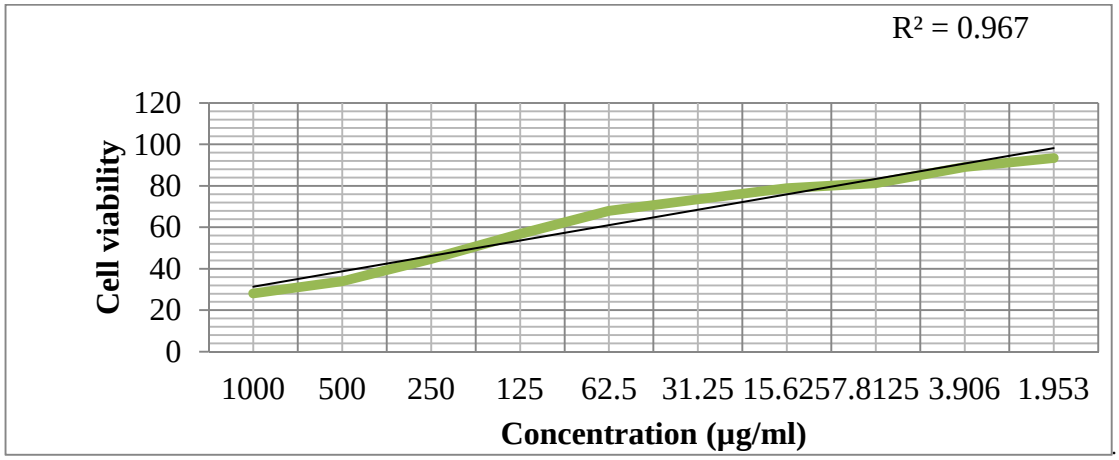
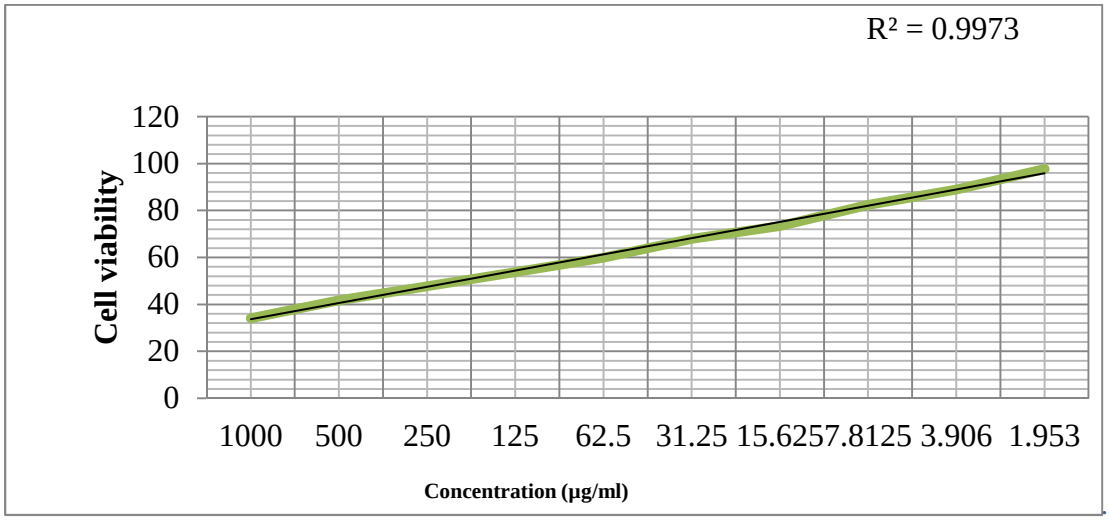


Fig – 2- MTT Assay for compound (2)



Fig– 3- MTT Assay for compound (3)



CONCLUSION

In summary, we are reporting the first instance of the synthesis, antibacterial and antifungal studies of the novel heterocyclic compounds. The antibacterial screening data of compounds (**1, 2 & 3**) shows very good antibacterial

activity against *V. cholera and K. pneumonia.*, whereas S *S. aureus, S. typi* and *E.faecalis* showed moderate inhibition zone against these compounds The antifungal studies of the novel heterocyclic compounds shows all the three organic compounds (**1,2 &3**) played significant role on pathogenic fungi *Aspergillus fumicaytus, Fusarium spp, Trichophyton rubrum and Penicillium spp*. The growth control effect was very high than the standard Amphotericin-B

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