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In view of the broad physiological activity of organometallic and organophosphorus compounds, the present paper deals with the synthesis of 2-(4-methoxybenzylidene amino) benzene-thiol, its metallation with mercury (II), nickel (II), palladium (II), and phosphorylation. The antimicrobial activities of all products were investigated. The palladated product displays a significant anticancer activity against human breast carcinoma cell. All the new products were investigated and their structures were elucidated using elemental analyses, thermal analysis, and spectroscopic data.

Keywords anticancer activity, antimicrobial, mercuration, metallation, palladation, phosphorylation, Schiff base

INTRODUCTION

Schiff bases occupy a special place in the realm of natural and synthetic organic chemistry because many products that contain this subunit exhibit useful and diverse biological activity. Many Schiff bases also have wide applications in several fields such as fluorescence properties in acidic media and for spectrofluorimetric monitoring of small pH change,[1] and biological inorganic and analytical chemistry.[2–6] Some Schiff bases acquire excellent characteristics and structural similarities with natural biological substances. Simple preparation, procedures, and synthetic flexibility enable design of suitable structural properties.[7,8] Some new Schiff bases were synthesized via the reaction of 1,3-propenediamine with isatin, and the reaction was found to proceed according to the mode of addition to afford two products as well as two isomers, followed by their metallation.[9] A series of Schiff base of Dapsone and their derivatives exhibited potent antibacterial activity.[10] Schiff bases derivatives of 4-methylpyridin-2-amine acquire antimicrobial activities.[11] The synthesis of some metallated Schiff bases with Ni (II), Co (II), and Cu (II) were found to be killing agents for Biomphalaria alexandrina snails without affecting the surrounding environment.[12] More et al.[13] marked the biological activity of Schiff bases synthesized from aminothiazoles. Azomethines exhibit a wide range of pharmacological activities like antimicrobial,[14] antiparasitic,[15] anti-inflammatory,[16] anticancer,[17] etc. Biological activities of some palladium(II) and platinum(II) compounds derivatives of 1-H-indol-2,3-dione benzothiazoline and 5- nitro-1-H-indol-2,3-dione benzothiazoline were found greater than the parent benzothiazolines towards a variety of fungal and bacterial strains.[18] The 4-Thiazolidinone derivatives of phenophosphazines have been evaluated as antibacterial and antifungal.[19] In this work, Schiff base and its metallated and phosphorylated products were synthesized and their biological activities were also studied, as well as the anticancer activity of the palladated product.

EXPERIMENTAL

Melting points were taken on a Gallen Kamp melting point apparatus and were uncorrected. Thin layer chromatography was performed with fluorescent silica gel plates HF_{254} (Merck), and plates were viewed under UV_{254} and 265 light. Elemental analyses were performed by The Microanalytical Center, Cairo University. Analyses of mercury were carried out by thiocyanate method[20] using ferric alum as indicator or by mercurometry using diphenyl carbazone-bromothymol blue as indicator.[21] Infrared spectra (FT-IR) were recorded on Bruker Vector Germany and on Mattson FT-IR 1000, using KBr disks. Mass spectra are measured on GCQ Finnigan MAT. ^{1}H-NMR spectra were recorded on Gemini-200 MHZ NMR spectrometer in DMSO-d_6 spectra were internally referenced to TMS. The antibacterial activity were determined in microanalytical center Cairo University, and anticancer activity was done in National Cancer Institute, Cancer biology department, Pharmacology, Cairo University.

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Preparation of Schiff Base 2-(4-methoxybenzylidene amino) Benzenethiol

A mixture of 2-aminothiophenol (0.125g, 1mmol) and (0.136g, 1mmol) of 4-methoxybenzaldehyde in 40 ml ethyl alcohol was refluxed for 4 hrs. The precipitated product was filtered, dried, and crystallized from ethyl alcohol to give compound (1). White crystals, 98% yield, m.p: 120–121°C. I.R ν (cm^{-1},KBr): 3059(C-H, arom.), 2992–2833(C-H, aliph.str.), 2559 (S-H), 1598(C=N), 1475(C-H aliph.bend.), 1225 (C-O-C Asymm. str.), 1024 (C-O-C sym. str).[22] ^{1}H-NMR (DMSO-d_6)δ: 8.1 (1 H, -HC=N), 8-7.1 (8H, Ar. protons), 3.8 (3H, OCH_3), 3.3 (1H, SH). Anal. calcd.for $C_{14}H_{13}NOS$: C 69.13, H 5.349, N 5.76, S 13.16, Found: C 69.00, H 5.32, N 5.74, S 13.13.

Metallation of (1) to give (2), (3) and (4) (General Procedure)

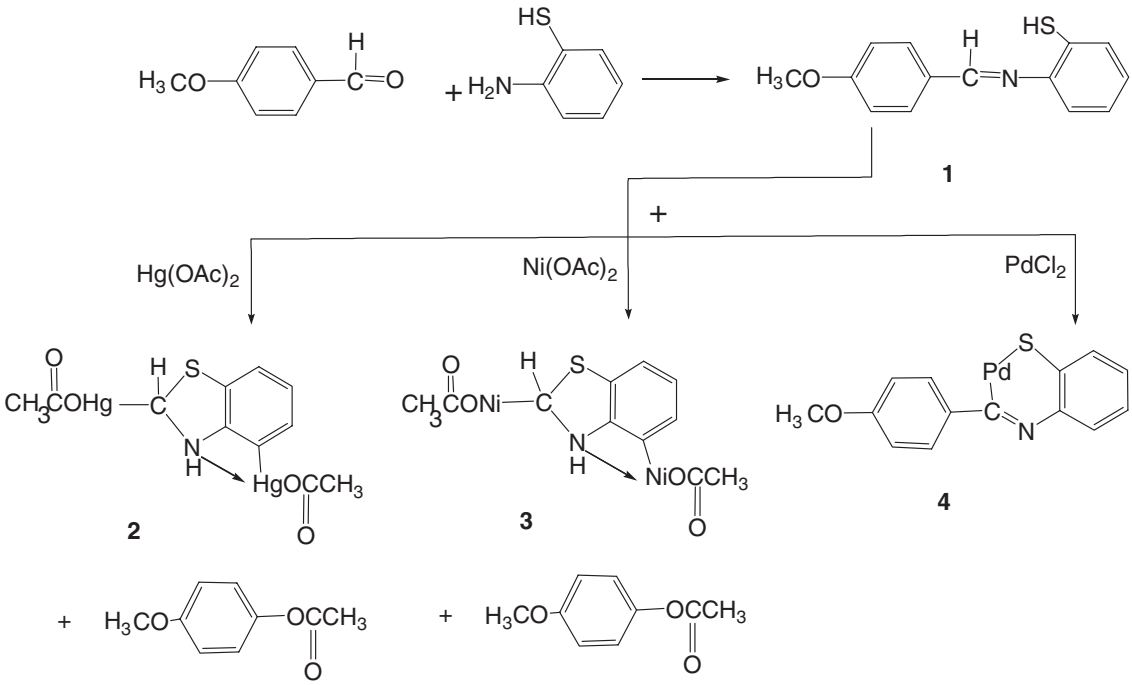
A mixture of (0.243 g, 1mmol) of Schiff base (1) and (1 mmol) of mercuric acetate or nickel acetate in 30 ml toluene and 2 ml acetic acid or palladium chloride in methanol and few drops of HCl was refluxed for 3 hours, left to cool where crystalline products (2), (3), or (4) were precipitated after cooling, filtered, dried, and crystallized from acetic acid to give (2) or (3) and from methanol to give compound (4). The filtrate for the product (2) and (3) was evaporated under reduced pressure and the residue (oily part) was detected as anisyl acetate.

Product (2): 75% yield, m.p: 240–241°C. IR (νcm^{-}1,KBr): 3210(NH), 3039(C-H, arom.), 2950- 2834(C-H, aliph. str.), 1475 (C-H aliph. bend.), 622 (C-S), 510 (Hg-C)[23]. MS m/z(%):241(100)$^+$HgNCO,243(9.2)$^+$HgCOCH$_3$,228(4.6)$^+$Hg-CH=N,226(57.6)$^+$HgCN, (198,199, 200, 201 for Hg^+),439(1.5) $C_{11}H_{12}NO_3SHg^+$. Anal. calcd. For $C_{11}H_{11}NSO_4Hg_2$: C 20.17, H 1.68, N 2.14, S 4.89, Hg 61.32. Found: C 20.00, H 1.66, N 2.12, S 4.84, Hg 60.80.

Anisyl acetate**:** Its b.p = 139°C/10mmHg, IR (νcm^{-1}, KBr):3046(C-H, arom.), 2954-2878(C-H, aliph.str.), 1742 (C=O, ester), 1370(C-H,aliph.bend.),1219(C-O-C Asym.str.),1018 (C-O-C Sym.str.) Anal. calcd. for: $C_9H_{10}O_3$: C 65.06, H 6.02. Found: C 65.00, H 5.90.

Metallated product (3): Green crystals, 76% yield, m.p: 340-341°C. I.R (νcm^{-1}, KBr)3641-3400(O-H), 3316-3212(NH), 3040(CH, arom.), 2997- 2935 (C-H, aliph. Str.), 1732 (C=O), 1410(C-H, aliph.bend.), 662 (C-S), 517 (Ni-C). MS spectrum m/z (%): M^+ 514(30), the base peak at 60 (100) $^{60}Ni^+$ or $CH_3COOH^{+\cdot}$. Anal. calcd. for $C_{11}H_{11}NSO_4Ni_2.8H_2O$: C 25.66, H 5.24, N 2.72, S 6.22, Ni 22.82. Found: C 25.60, H 5.22, N 2.71,S 6.20, Ni 22.74.

Palladated product (4): Orange crystals, 85% yield, m.p. above 330°C. IR (νcm^{-1}, KBr): 3040(C-H, arom.), 2912–2841(C-H,aliph.str.), 1598(C=N), 1440(C-H, aliph. bend.), 1219 (C-O-C asymm. str.), 1018 (C-O-C symm. str.), 431 (Pd-C). MS spectrum m/z(%): the molecular ion peak at m/z 491 (0.1) and the base peak at m/z 241 (100)$C_{14}H_{12}NOS^+$. ^{1}H-NMR (DMSO-d_6)δ (ppm): 8-7.1 (8H, arom), 3.8 (3H, OCH_3).Anal. calcd. for $C_{14}H_{11}NSO$ Pd.HCl. 3 1/3 CH_3OH: C 42.44, H 5.16, N 2.86, S 6.53 Pd 21.75 Found: C 42.22, H 5.14,N 2.83,S 6.48, Pd 21.50.



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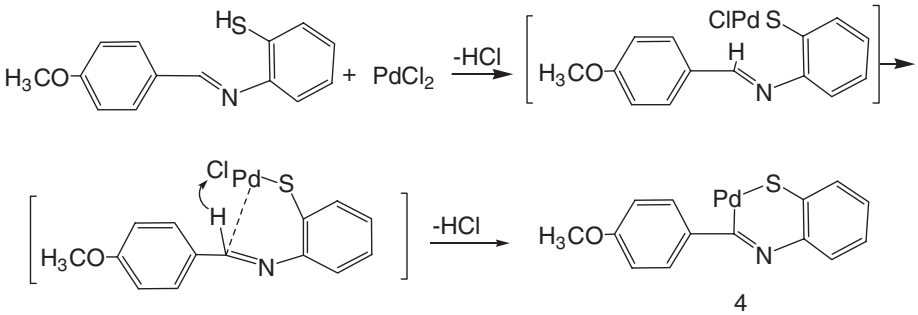

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Phosphorylation of (2), (3), and (4) to give (5), (6), and (7) (General Procedure)

A mixture of (1mmol) of (2) or (3) or (4) and (1mmol) of triphenylphosphine in 30 ml tetrahydrofuran was refluxed for 3 hrs, the precipitate formed was filtered, dried, and crystallized from petroleum ether 40–60.

Phosphorylated product (5)**:** Pale blue crystals, 30% yield, m.p: decomposed at 288°C. IR (ν cm^{-1}, KBr): 3056(C-H, arom.), 1433 (P–Ph), 524 (Hg-C). Anal. calcd. for $C_{61}H_{48}NSP_3Hg$: C 65.38, H 4.28, N 1.25, S 2.85, P 8.30, Hg 17.91. Found: C 65.50, H 4.29, N 1.25, S 2.86, P 8.32, Hg 17.94.

Phosophorylated product (6)**:** Greenish- white crystals, 90% yield, m.p: 240-241°C. IR (ν cm^{-1}, KBr): 3340-3210(NH),3040(CH, arom.), 2997- 2935 (C-H, aliph. Str.), 1732 (C=O), 1470(CH, aliph.bend.), 1433 (P-Ph), 662 (C-S), 530(Ni-C),. MS spectrum m/z(%): the base peak at m/z 77 (100) $C_6H_5^+$, 92 (16.7) $C_6H_5NH]^+$, 109 (16.7) $C_6H_5S]^+$, m/z 60 (11.1), m/z 61 (13.9), m/z 62 (10) Ni isotopes, m/z 277 (88.2%) $Ph_3P=NH]^+$, m/z 278 (17.6) $Ph_3P=NH_2]^+$, m/z 395 (13.9) Ph_3PH-NH-NiOAc$]^+$. Anal. calcd. for $C_{29}H_{26}NSO_4PNi_2$: C55.02, H 4.11, N 2.21, S 5.06, P 4.90, Ni 18.56. Found: C 55.76, H 4.16, N 2.33, S 5.13, P 4.97, Ni 18.80.

Phosphorylated product (7): Orange crystals, 94% yield, m.p: 145–146°C. I.R (ν cm^{-1}, KBr): 3059(C-H, arom.), 2995- 2831(C-H, aliph.str.), 1598(C=N), 1475(C-H aliph.bend.), 1431 (P-Ph). 1225 (C-O-C Asymm. str.), 1024 (C-O-C sym. str). MS m/z(%) 51(100)$C_4H_3^+$, 65(10.6)$C_5H_5^+$, 77(60.2)$C_6H_5^+$, 103(4.4)$C_6H_5CN^+$, 107(33.6)$C_7H_7O^+$, 108(81.4)$C_6H_5S^+$, 133(4.4)$C_7H_4NS^{+\cdot}$, 183(63.7)$C_{12}H_9NO^+$, 185(13.3)PPh_2^+, 186(4.4)$C_{12}H_{12}NO^+$, 262(50.4)P-Ph_3^+ Anal. calcd. for $C_{32}H_{26}NSOP$. HCl. CH_3OH: C 69.29, H 5.42, N 2.44, P 5.42 Found: C 69.50, H 5.43, N 2.44, P 5.43.

RESULTS AND DISCUSSION

o-Aminothiophenol reacted with p-methoxybenzaldehyde to afford Schiff base 2-(4-methoxybenzylidene amino) benzenethiol (1).

Product (1) was allowed to react with some metal salts such as mercuric acetate, nickel acetate, and palladium chloride to obtain the metallated products. The overall metallation reactions are summarized as follows in Scheme 1.

The mechanism of the reaction with mercuric acetate may proceed as follows (Scheme 2):

The data obtained from IR, ^{1}H-NMR, MS, and elemental analysis was consistent with the proposed structure. The reaction of nickel acetate with compound (1) gave rise to product (3) and anisyl acetate. The IR spectra for products (2) and (3) showed new bands for ν_{Hg-C}, ν_{Hg-N}, ν_{C-S}, and ν_{Ni-C}, ν_{Ni-N}, and ν_{C-S}. The other product produced via bond cleavage was assigned to be anisyl acetate according to its boiling point, IR, and elemental analysis. The structures were confirmed also considering the data obtained from^{1}H-NMR and MS spectra.

Compound (4) may form via the following suggested mechanism:

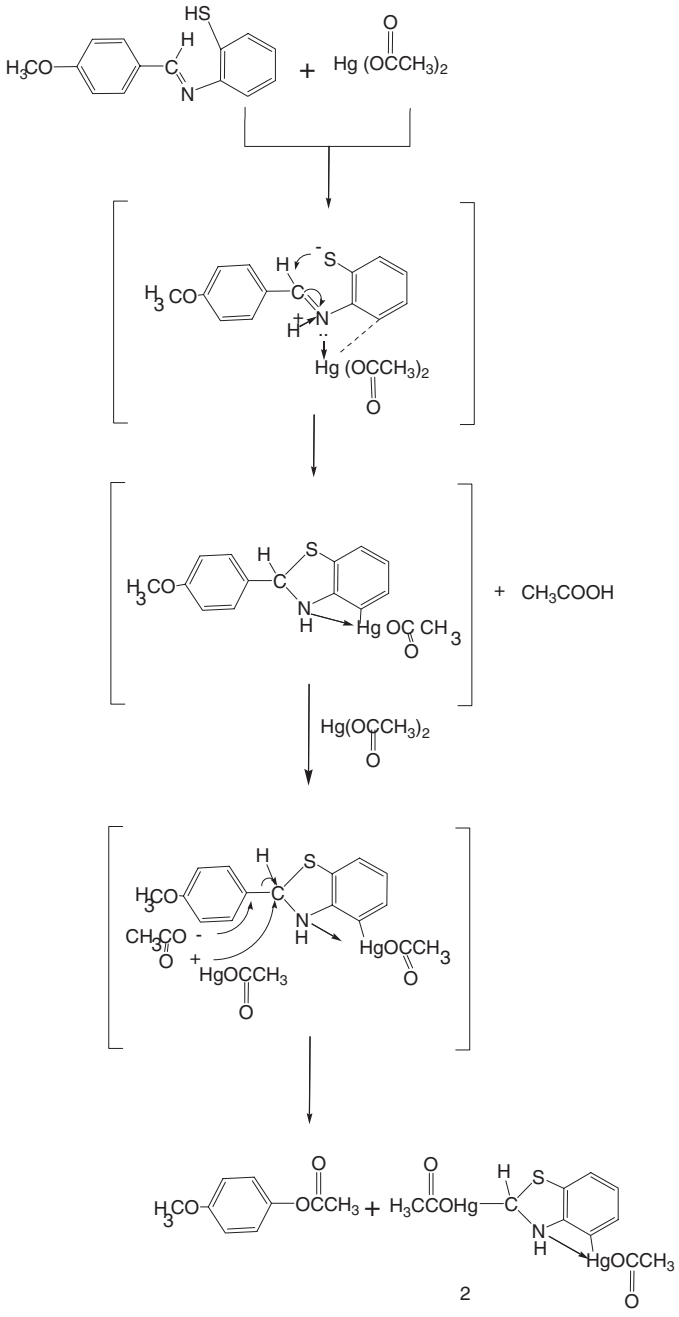
All the data obtained confirm the structure of (4). The ^{1}H-NMR spectrum shows δ 8.0–7.12 ppm for aromatic protons(8H), and δ 3.88 ppm for OCH_3(3H).

Measurement of Potential Cytotoxicity of Compound (4) by SRB Assay

Potential cytotoxicity of the compound was tested using the method of Skehan.[24] Cells were plated in 96-multiwell plate (10^4 cells/ well) for 24 hrs before treatment with the compound to allow attachment of cells to the wall of the plate. Different concentrations of the compound under test (0, 5, 12.5,

TABLE 1
In vitro cytotoxic activity of the newly synthesized compound (4), in comparison to 5-Flurouracil and Doxorubicin

Conc. μg/ml	MCF7-4	MCF7-5-Flurouracil	MCF7-Doxorubicin
0.000	1.000	1.000	1.000
5.000	0.4158	0.3369	0.1942
12.50	0.2275	0.1818	0.1717
25.00	0.1825	0.0883	0.1855
50.00	0.2366	0.0880	0.2013
IC50 μg/ml	5.43	4.437	4.165



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SCH. 2.

25, 50 μg /ml) were prepared for each individual dose. Monolayer cells were incubated with the compound for 48 hrs at 37°C and in an atmosphere of 5% CO_2. After 48 hrs, they were fixed, washed, and stained with sulfo-rhodamine-B stain. Excess stain was washed with acetic acid and attached stain was recovered with tris base. Color intensity was measured in an ELIZA reader. The relation between surviving fraction and drug conc. was plotted (Table 1 and Figure 1) to get the survival curve of each tumor cell line after the specified compound. The antitumor activity result indicated that compound (4) showed

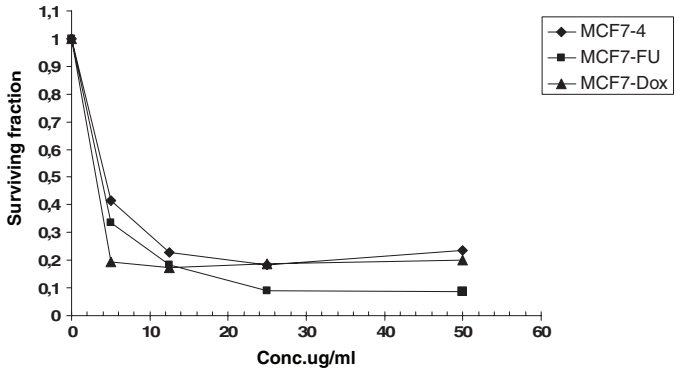


FIG. 1. Drug cytotoxicity of compound (4) , 5-flurouracil and Doxorubicin. IC50 for compound (4)= 5.43μg/ml; IC50 for flurouracil = 4.43 μg/ml; and IC50 for Doxorubicin= 4.17 μg/ml.

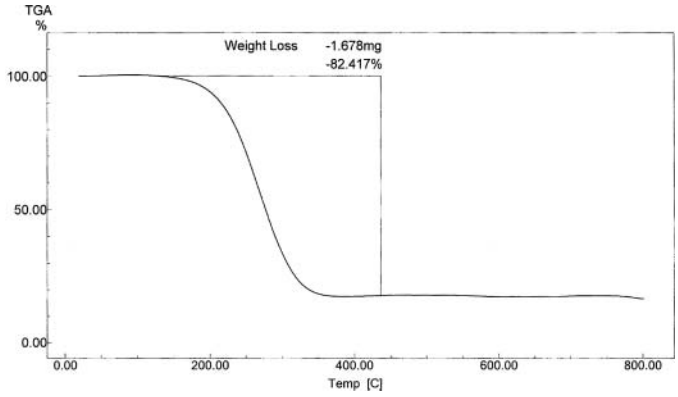


FIG. 2. TGA of phosphorylated product (5).

antitumor activity against the tested human breast carcinoma cell line MCF7 (in vitro) with varying intensities in comparison to the known anticancer drugs 5-Flurouracil and Doxorubicin. Moreover, compound (4) showed cytotoxic activity (IC50 equal 5.43 μg/ml) while 5-Flurouracil (IC50 equal 4.43 μg/ml) and Doxorubicin (IC50 equal 4.16 μg/ml). The reaction of triphenylphosphine with the metallated products (2-4) in THF under reflux for 3 hrs, afforded phosphorylated compounds (5), (6), (7).

The structures were elucidated considering elemental analyses, IR, MS, and thermal analysis of compound (7). The MS spectrum of compound (6) showed the base peak at m/z 77 can be attributed to $C_6H_5^+$ and m/z 277(88.26%) due to $Ph_3P{=}N^+$, m/z 395(13.6%) due to $Ph_3P{=}N\text{-}NiOAc^{+\cdot}$**.** Trials for reaction of triphenylphosphine with compound (1) in order to obtain compound (7) directly failed; this indicated that only in the presence of Pd in compound (4) facilitates the replacement by triphenylphosphine.

Structure identification of compounds (5), (7) by thermal analyses:[25]

The thermogravimetric analysis (TG) of the phosphorylated product (5) is shown in (Figure2). One step of weight

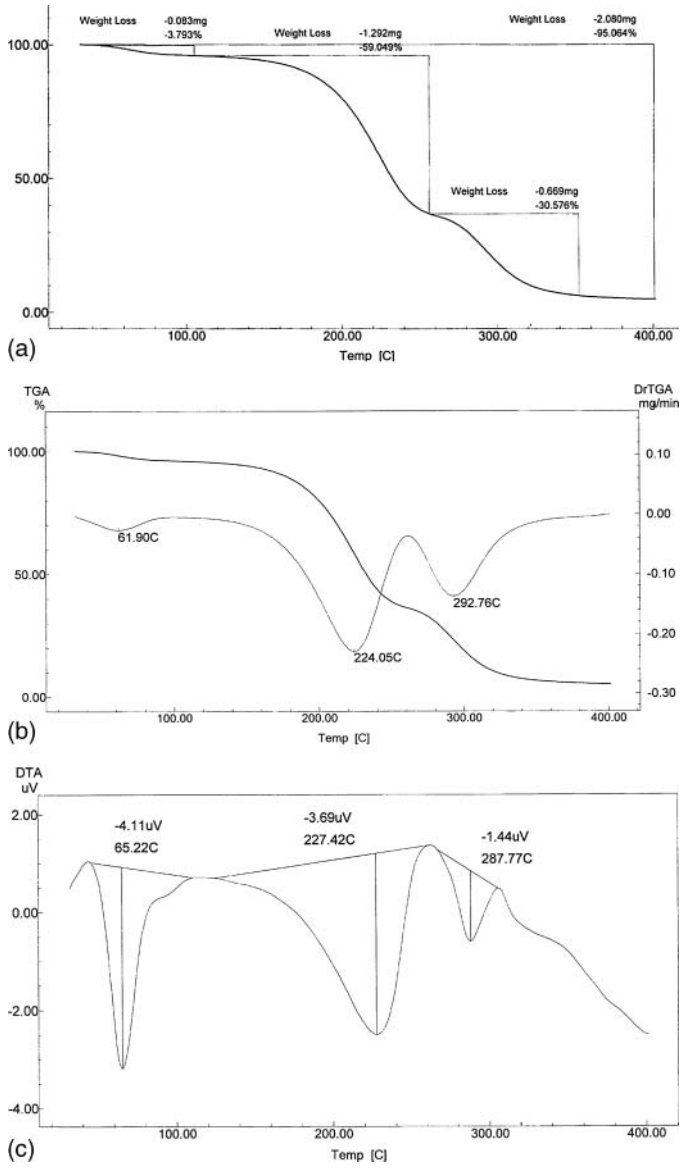


FIG. 3. (a) TGA of phosphorylated product (7); (b) DTGA of phosphorylated product (7); (c) DTA of phosphorylated product (7).

loss (82.41%) (calculated 82.08%), the remaining may be Hg (17.91%) or HgO (19.34%).

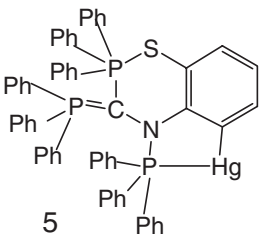
The thermogravimetric analysis (TG) of the phosphorylated product (7) is shown in Figure 2a, differential thermogravimetric (DTG) is shown in Figure 2b and differential thermal analysis (DTA) is shown in Figure 2c. Three steps of weight loss; the first methyl group by 3.7% (calculated 3.0%) then the second step loss of triphenylphosphine sulphide $Ph_3P{=}S$ with weight loss 59% (calculated 58.45%), the third one loss by 30.6% loss of all the remaining organic compound. This indicated also by 3 endothermic peaks at 65.22, 227.42, and 287.77°C, respectively.

Measurement of Antimicrobial Activity Using Diffusion Disc Method

A filter paper sterilized disc saturated with measured quantity of the sample was placed on plate containing solid bacterial medium (nutrient agar broth) or fungal medium (Doxs medium) that has been heavily seeded with the spore suspension of the tested organism. After inoculation, the diameter of the clear zone of inhibition surrounding the sample is taken as a measure of the inhibitory power of the sample against the particular test organism.[26–29] The antimicrobial activity of all compounds were tested and the results showed that compound (1) has no activity, while the rest compounds acquire antimicrobial activity towards some types of bacteria as shown in Table 2. The order of increasing activity is (5)>(2)>(3)>(6)>(7)>(4).

CONCLUSION

2-(4-methoxybenzylidene amino) benzenethiol (1) was synthesized, metallated using mercuric acetate, nickel acetate to afford dimetallated products (2),(3) accompanied by bond cleavage, with the liberation of anisyl acetate, and with palladium chloride to form monopalladated product (4).The suggested reaction mechanisms are given. The metallated products reacted with triphenylphosphine to give tri-phosphorylated product (5) and phosphorylated 6-membered hetero ring products (6), (7) with the loss of palladium metal (product **7**). The elucidation of the structures based on IR, MS, and elemental analyses. Compound 7 studied by thermogravimetric analysis. The antimicrobial activity of all compounds were tested and the results showed that compound (1) has no activity, while the rest compounds exhibit antibacterial activities towards



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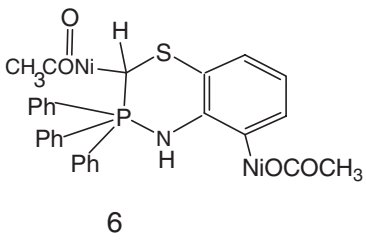

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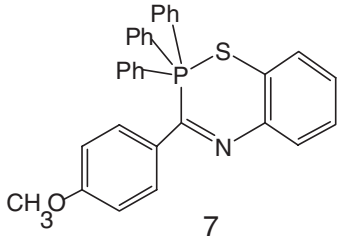

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TABLE 2
Antimicrobial activity of the products towards some types of bacteria

	Inhibition zone diameter(mm/ mg sample			
Sample	*Escherichia coli* (G^-)	*Pseudomonas aeruginosa* (G^-)	*Staphylococcus aurous* (G^+)	*Streptococcus faecalis* (G^+)
(1)	0.0	0.0	0.0	0.0
(2)	14	39	13	37
(3)	19	17	28	16
(4)	15	15	14	14
(5)	35	35	36	32
(6)	16	15	18	16
(7)	13	16	15	16

Escherichia coli, *Pseudomonas aeruginosa*, *Staphylococcus aurous*, and *Streptococcus faecalis*. The order of increasing activity is (5)>(2)>(3)>(6)>(7)>(4). However, product (4) was found to have anticancer activity compared with the known 5-Flurouracil and Doxorubicin.

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