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SYNTHESIS AND EVALUATION OF ANTICANCER ACTIVITY OF SOME NEW SCHIFF BASES OF AMINO-THIOPHENE DERIVATIVES

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ABSTRACT

A new series of amino-thiophene Schiff bases **(IIa-i)** was synthesized and characterized using spectroscopic methods. Schiff bases were prepared via condensation reactions of different aromatic aldehydes and amino-thiophene. All Schiff bases were tested against MCF7 and HCT116 cell lines, the imine compounds exhibited selectivity excellent cytotoxic for aforementioned carcinoma cell lines. Especially, compound **IIc** and **IIh** were the most active compounds against both cell lines, it is well known that, the difference in the characteristics of substituents attached to the Schiff base function leads to interesting variations within pharmacological actiivty of resultant molecular systems.

KEYWORDS: Schiff Bases, Anti-cancer activity, Thiophene.

1. INTRODUCTION

Cancer is the second highest cause of death in industrialized countries.[1,2] Despite the diversity of drugs, cancer often proves incurable due to the development of drug resistance.[2] With >100 types of cancer exist, 8.2 million people die each year from cancer, an estimated 13% of all deaths worldwide and 70% the increase in new cases of cancer expected over the next 2 decades.[3] Schiff bases, are known as azomethine or imine as nitrogen analogue an aldehyde or ketone structurally, where imine or azomethine group found to replace the carbonyl group.[4] Schiff bases or imines, products of the condensation of carbonyl compound and primary amines, are important molecules that have been extensively studied owing to their broad range of industrial and biomedical applications.[5] Schiff base is very widely used and the most appreciated organic building blocks to have a diverse range of pharmacological importance as antioxidant, anthelmintic, anti-tubercular, anti-inflammatory, antimicrobial and anticonvulsant.[6] Imines have also been found to possess cytotoxic and antiproliferative activity towards several cancer cell lines like leukemia, colorectal adenocarcinoma (Caco-2), and pancreatic cancer (Panc-1)[7-8], where the presence of the azomethine bond (CH=N) is believe to be critical to biological activity.[6,9] Azomethine's nitrogen atom are supposed to get interacted with active centers of cell constituents via forming a hydrogen bond which interferes with normal cell processes and results in the destruction of enzymatic activity of cancerous cells, thereby presents Schiff base as a potential target to discover anticancer agents.[10] In cancer, thiophene derivatives have been shown to exhibit cytotoxicity in several type of cancer cells, including, leukemia, ovarian, glioma, renal, breast and colon.[11-14] Hence, we have selected thiophene ring as a starting heterocyclic core to build the desire Schiff bases, such heterocyclic compounds have a considerable active role as antitumor agents.[11,12,15,16] Based on the above findings, the aim in this research is to develop a novel series of Schiff's bases containing thiophenes with potential anti-cancer activity.

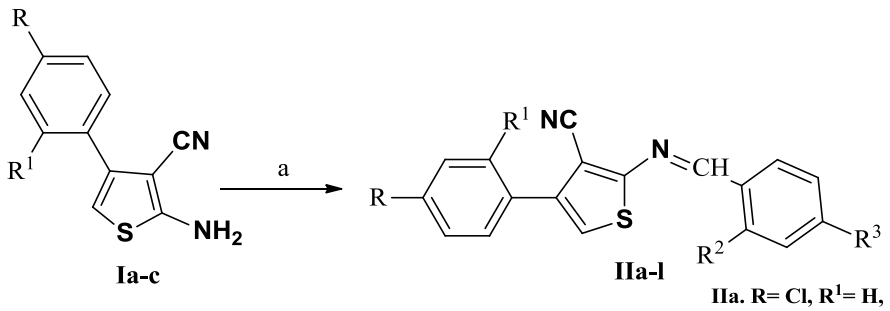
2. RESULTS AND DISCUSSION

2.1. Chemistry

Scheme 1 represents the chemical steps followed to obtain desired Schiff base **Ia–i**. Knoevenagel condensation of substituted acetophenone with malononitrile afforded substituted, 2-[1-(aryl)-ethylidene]-malononitrile which upon base promoted cyclization with elemental sulfur gave substituted 2-aminothiophene-3- carbonitrile **Ia-c** in excellent yield[10-11], which was selected as a starting material to be treated with various desired aromatic aldehydes.

The synthesis of the target compounds **IIa-i** was accomplished in good yields via the reaction of the substituted 2- aminothiophene-3- carbonitrile **Ia-c** with the appropriate aldehyde in ethanol (Scheme 1). Structure of compounds **IIa-i** was established on the bases of spectral data. IR spectra showed absorption bands at 3039 and 3183 cm^{-1} for aromatic C-H and at 1591 and 1691 cm^{-1} for azomethine group (-CH=N-). Formation of Schiff bases was confirmed by the absence of characteristic IR absorption peak (3140-3400 cm^{-1}) of N-H stretching, due to conversion of -NH2group to -CH=N-group. The ^{1}H NMR showed sharp singlet peak in the range of δ 8.20-8.47 ppm indicating the presence of azomethine proton (-CH=N-). The multiplet at δ6.60-8.65 ppm was due to aromatic protons. Also the appearance of signals at 2.30, 2.83, 3.90, 2.40, 3.80, 2.20, 2.30 and 2.35 ppm confirmed the presence of methyl groups for **IIa, IIe, IIf, IIg, IIi, IIj, IIk and IIl**.

Scheme 1



Ia R= Cl, R^1= H
Ib R = NO_2, R^1= H
Ic R = $OCOCH_3$, R^1= OH

IIa. R= Cl, R^1= H, R^2= H, R^3= CH_3
IIb. R= Cl, R^1= H, R^2= H, R^3= Cl
IIc. R= Cl, R^1= H,,R^2= NO_2, R^3= H
IId. R= Cl, R^1= H,, R^2= NO_2, R^3= NO_2
IIe. R= Cl, R^1= H, R^2= H, R^3= N,N,dimethyl
IIf. R= Cl, R^1= H, R^2= H, R^3=OCH_3
IIg. R= NO_2, R^1= H, R^2= H, R^3= CH_3
IIh. R= NO_2, R^1= H, R^2= H, R^3= Cl
IIi. R= NO_2, R^1= H,, R^2= H, R^3= OCH_3
IIj. R= $OCOCH_3$ R^1= OH, R^2= H, R^3= H
IIk. R= $OCOCH_3$ R^1= OH, R^2= H, R^3= Cl
IIl. R= $OCOCH_3$ R^1= OH, R^2= H, R^3= NO_2

Reagents and conditions: a-Aromatic aldehydes, Ethanol, reflux

2.2. Biological studies

The newly synthesized compounds Schiff base **IIa-l** were subjected to the screening of their cytotoxic activity against human breast cancer cell line MCF-7 and colon cancer HCT116 cell line using Sulforhodamine B colorimetric assay (SRB assay).[16-17] The IC50 values of the tested compounds are summarized in Table 1 and the bioassay data suggested that these new Schiff base displayed a variable degree of anticancer activity.

Thiophene ring was noticed to have an essential role in cancerous cell inhibitory effects, whereas, varying the type of coupled aromatic entity to the Schiff base function has a key role in determining the final anticancer activity of resultant molecule.

Compound **IIc** and **IIh;** R= choro and nitro substitution (electron-withdrawing groups) showed the highest anticancer activity against MCF-7 cell line with IC_{50} 11.2 and 15.1 µg/ml respectively, while Compounds **IIc** and **IIl** showed the highest anticancer activity against against colon cancer cell line with 21.5 and 15 µg/ml respectively.

Table 1: Results of in vitro cytotoxic activity of tested compounds on breast cancer cell line (MCF-7) and colon cancer cell line (HCT 116).

Cpd. No	MCF-7, IC_{50}, (µg/mL)	HCT116 IC_{50} (µg/ml)
IIa	34	31.3
IIb	>50	>50
IIc	15.1	21.5
IId	>50	36
IIe	23	18
IIf	49	39.9
IIg	41	36.8
IIh	11.2	25.9
IIi	41	38.5
IIj	>50	>50
IIk	48	20.5
IIl	37	15

3. CONCLUSION

In brief, a new series of amino-thiophene Schiff bases **(IIa-l)** was prepared and characterized by spectroscopic methods. Schiff bases were synthesized by condensation reactions of benzaldehyde derivatives and amino-thiophene. All Schiff bases were validated against MCF7 and HCT116 cell lines by Sulforhodamine B colorimetric assay (SRB assay). According to the results, all of the imine compounds exhibited selectivity for the selected carcinoma cell lines. Especially, compound **IIc** and **IIh** were the most active compounds against both tested cell lines, it is known that electron withdrawing aromatic substituents like halogens and nitro groups enhance cytotoxic activity, and their para position highlight compound **IIc** and **IIh** as a strong anticancer agent. Moreover, many of the final Schiff base derivatives showed<50 ug/ml of IC_{50} against two cancerous cell lines.

All thiophene derivatives showed cytotoxic activity against MCF-7 and HCT116 cells. It was noticed that thiophens ring has an essential role to provide promising cancerous cell inhibitory effects, whereas, varying the type of coupled aromatic rings to the Schiff base function has a key role in determining the final anticancer potency of resultant compound.

4. Experimental

4.1. Chemistry

Chemistry Melting points (mp) were determined on Stuart apparatus and the values given are uncorrected. IR spectra were determined on Shimadzu IR 8400 s spectrophotometer (KBr, cm−1). ^{1}H-NMR spectra were carried out using a Mercury, a Gemini 300-BB 300 MHz and Joel (eca) 500 MHz spectrophotometers using tetramethylsilane (TMS) as internal standard. Chemical shift values were recorded in ppm on δ scale, The solvent system was benzene, chloroform and methanol with different ratios and spots were visualized using UV lamp.

Evaluation of the cytotoxic activity was performed at the Egyptian National Cancer Institute. Compounds **Ia&b** were synthesized according to reported method.[11]

Acetic acid 4-(5-amino-4-cyano-thiophen-3-yl)-3-hydroxy-phenyl ester (Ic)

Elemental sulfur (1.2 mmol) and Acetic acid 4-(2,2-dicyano-1-methyl-vinyl)-3-hydroxy-phenyl ester (1 mmol) were suspended in tetrahydrofuran (10 mL) and warmed in water bath to 35 ºC. A solution of sodium bicarbonate (10%, 5mL) was added over 1 h with maintaining the reaction temperature below 65ºC. The reaction mixture was stirred at 35 ºC for 30 min then sodium chloride solution (12.5%, 20mL) was added in one portion and the organic layer that separated was successively washed with sodium chloride solution (25%, 12 mL ×3). The organic layer was then concentrated under reduced pressure to give the crude product that was crystallized from appropriate solvent.

Yield, 66% (acetone). mp: 240-242 ^{o}C. IR (KBr, cm^{-1}): ***v*** = 3350 (broad OH), 2219 (CN), 1747 C=O). ^{1}H NMR (DMSO) ppm 2.80 (s, 3H, CH_3), 6.97 (s, H, thiophene), 7.38 (s, 2H, NH) 7.50-8.10 (m, 3H, ArH), 10.00 (s,1H, OH) ppm.

General procedure of preparation of Schiff-base II (a-l)

A mixture of **Ia-c** (1 mmol) and the appropriate aromatic aldehyde (1 mmol) in absolute ethanol (20 mL) was heated under reflux for 6h. The reaction mixture was cooled and the formed solid was filtered, dried and crystallized from appropriate solvent.

4-(4-Chlorophenyl)-2-((4-methylbenzylidene) amino) thiophene-3-carbonitrile (IIa)

Yield, 70% (ethanol). mp = 162-164^{o}C .IR (KBr, cm^{-1}): *v* =3039 (CH, aromatic) 2973 (CH, aliphatic) 2222 (CN), 1620 (C=N),^{1}HNMR (DMSO) ppm: 2.35 (s,3H, CH_3),6.90-7.20 (m, 4H, ArH),7.60 (s,1H, thiophene), 7.70-7.85 (m, 4H, ArH), 8.60 (s, 1H, H-C=N).

2-((4-Chlorobenzylidene) amino)-4-(4-chlorophenyl) thiophene-3-carbonitrile (IIb)

Yield, 60% (ethanol). mp = 198-200^{o}C. IR (KBr, cm^{-1}): *v* = 3069 (CH, aromatic) 2220 (CN), 1622 (C=N), 1450 (C=C), ^{1}HNMR (DMSO) ppm:7.15-7.30 (m,

4H,ArH),7.65 (s,1H, thiophene), 7.70-7.95 (m, 4H, ArH), 8.55 (s,1H, H-C=N).

4-(4-Chlorophenyl)-2-((2-nitrobenzylidene) amino) thiophene-3-carbonitrile (IIc)
Yield, 70% (ethanol). mp = 230-232^{o}C. IR (KBr, cm^{-1}): *v* =3069 (CH, aromatic), 2222 (CN), 1625 (C=N),1435 (C=C),^{1}HNMR (DMSO) ppm:7.35-7.45 (m, 4H, ArH),7.50 (s,1H, thiophene), 7.60-7.70 (m, 2H, ArH), 7.90-8.10 (m, 2H, ArH), 8.41 (s,1H, H-C=N).

4-(4-Chlorophenyl)-2-((2,4-dinitrobenzylidene) amino) thiophene-3-carbonitrile (IId)
Yield, 70% (ethanol). mp = 150-152^{o}C. IR (KBr, cm^{-1}): *v* = 3076 (CH, aromatic), 2222 (CN), 1616 (C=N),1439 (C=C), ^{1}HNMR (DMSO) ppm:7.33-7.42 (m, 4H, ArH),7.42 (s,1H, thiophene), 7.82-7.93 (m, 2H, ArH), 8.40 (s, 1H, ArH), 8.59 (s,1H, H-C=N).

4-(4-Chlorophenyl)-2-((4-(dimethylamino) benzylidene) amino) thiophene-3-carbonitrile.(IIe)
Yield, 90% (ethanol). mp = 229-231^{o}C. IR (KBr, cm^{-1}): *v* = 3049 (CH, aromatic), 2980 (CH, aliphatic) 2224 (CN), 1611 (C=N),1430 (C=C),^{1}HNMR (DMSO) ppm: 3.25 (s,6H,2CH$_3$), 6.90-7.30 (m,4H,ArH),7.50 (s,1H, thiophene), 7.65-7.70 (m, 4H, ArH), 8.42 (s, 1H, H-C=N).

4-(4-Chlorophenyl)-2-((4-methoxybenzylidene) amino) thiophene-3-carbonitrile (IIf)
Yield, 80% (aceton). mp = 194-196^{o}C. IR (KBr, cm^{-1}): *v* = 3069 (CH, aromatic), 2975 (CH, aliphatic) 2220 (CN), 1610 (C=N),1420 (C=C), ^{1}HNMR (DMSO) ppm: 3.90 (s,3H,CH$_3$), 6.81-7.30 (m,6H,ArH), 7.56 (s,1H, thiophene), 7.62-7.78 (m, 2H, ArH), 8.56 (s, 1H, H-C=N).

2-((4-Methylbenzylidene) amino)-4-(4-nitrophenyl) thiophene-3-carbonitrile (IIg)
Yield, 50% (ethanol). mp =200-202^{o}C. IR (KBr, cm^{-1}): *v* =3088 (CH,aromatic) 2229 (CN), 1606 (C=N),1450 (C=C),^{1}HNMR (DMSO) ppm: 2.41 (s,3H,CH$_3$),6.90-7.20 (m, 4H,ArH),7.21 (s,1H, thiophene), 7.50-7.85 (m, 2H, ArH), 8.14-8.23(m, 2H, ArH), 8.50 (s, 1H, H-C=N).

2-((4-Chlorobenzylidene) amino)-4-(4-nitrophenyl) thiophene-3-carbonitrile (IIh)
Yield, 46% (ethanol). mp =228-230^{o}C. IR (KBr, cm^{-1}): *v* =3097 (CH,aromatic) 2225 (CN), 1610 (C=N), 1420 (C=C), ^{1}HNMR (DMSO) ppm: 7.10-7.30 (m,4H,ArH),7.29 (s,1H, thiophene), 7.40-7.64 (m, 2H, ArH), 7.88-7.97 (m, 2H, ArH), 8.47 (s, 1H, H-C=N).

2-((4-Methoxybenzylidene) amino)-4-(4-nitrophenyl) thiophene-3-carbonitrile (IIi)
Yield, 60% (aceton). mp = 266-268^{o}C. IR (KBr, cm^{-1}): *v* =3091 (CH, aromatic), 2995 (CH, aliphatic) 2220 (CN), 1610 (C=N), 1420 (C=C),^{1}HNMR (DMSO) ppm: 3.80 (s,3H,CH$_3$), 6.98-7.30 (m,4H,ArH), 7.47 (s,1H, thiophene), 7.71-7.88 (m, 4H, ArH), 8.60 (s, 1H, H-C=N).

4-(5-(Benzylideneamino)-4-cyanothiophen-3-yl)-3-hydroxyphenyl acetate (IIj)
Yield, 41% (aceton). mp = 92-94^{o}C. IR (KBr, cm^{-1}): *v* = 3080 (CH,aromatic)2980 (CH, aliphatic) 2400 (CN), 1620 (C=N), 1422 (C=C), ^{1}HNMR (DMSO) ppm: 2.10(s,3H,CH$_3$),6.66 (s,1H,ArH), 7.20 (s,1H, thiophene), 7.40-7.58 (m, 2H, ArH), 7.60-7.74 (m, 5H, ArH), 8.53 (s, 1H, H-C=N), 9.00 (s,1H,OH).

4-(5-((4-Chlorobenzylidene) amino)-4-cyanothiophen-3-yl)-3-hydroxyphenyl acetate (IIk)
Yield, 44% (aceton). mp = 90-92^{o}C. IR (KBr, cm^{-1}): *v* = 3090 (CH, aromatic), 2950 (CH, aliphatic) 2410 (CN), 1620 (C=N),1428 (C=C),^{1}HNMR(DMSO)ppm: 2.12 (s,3H,CH$_3$), 2.30 (s,3H,CH$_3$), 6.84(s,1H,ArH),7.36 (s,1H, thiophene), 7.30-7.58 (m, 2H, ArH), 7.50-7.64 (m, 4H, ArH), 8.64 (s, 1H, H-C=N), 9.12 (s,1H,OH).

4-(4-Cyano-5-((4-nitrobenzylidene) amino)thiophen-3-yl)-3-hydroxyphenyl acetate (IIl)
Yield, 44% (aceton). mp = 97-99^{o}C. IR (KBr, cm^{-1}): *v* =3079(CH, aromatic) 2990 (CH, aliphatic)2430(CN), 1629(C=N), 1414(C=C), ^{1}HNMR(DMSO)ppm:, 2.35(s,3H,CH$_3$), 7.01(s,1H,ArH), 7.22(s,1thiophene), 7.29-7.41 (m, 2H, ArH), 7.807.90(m, 4H, ArH), 8.64 (s, 1H, H-C=N), 9.19(s,1H,OH).

4.3. Cytotoxic activity
The antitumor activity was determined for the newly synthesized compounds in Egyptian National Cancer Institute (NCI) for *in vitro* detection of IC$_{50}$ of their antitumor activity. The breast tumor cell line (MCF-7) was obtained frozen in liquid nitrogen (-180 ^{0}C) from the American Type Culture Collection (ATCC) and was maintained in the National Cancer Institute, Cairo, Egypt, by serial sub culturing. All chemicals used in this study are of high analytical grade. They were obtained from (either Sigma-Aldrich or Bio-Rad). The cytotoxicity of the test compounds was determined using.

SRB assay applying the method of Skehan et al.[17] These testing procedures were carried out at pharmacology lab at Cancer Biology Unit in Egyptian National Cancer Institute. The Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the compounds to allow the attachment of cells to the wall of the plate. Different concentrations of each compound (0, 1, 2.5, 5 and 10 mg/mL) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the compounds for 48 h at 37 ºC and in atmosphere of 5% CO2. After 48 h, cells were fixed, washed, and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction

and drug concentration was plotted to get the survival curve of each tumor cell line, The IC_{50} value was calculated using sigmoidal dose response curve-fitting models (Graph Pad, Prizm software incorporated), each concentration was repeated 3 times.

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