Hindawi Publishing Corporation
Journal of Chemistry
Volume 2013, Article ID 478635, 9 pages
http://dx.doi.org/10.1155/2013/478635

Research Article

Synthesis and Biological Evaluation of New N,N′-Bis(1-substituted-ethylidene)-ethane1,2-diamine and Their Acetyl and Trifluoroacetyl Derivatives as Cytotoxic and Antimicrobial Agents

Khalid A. Khan,[1] Hassan M. Faidallah,[1] and Abdullah M. Asiri[1,2]

[1] *Department of Chemistry, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia*
[2] *Center of Excellence for Advanced Materials Research, King Abdulaziz University, P.O. Box 80203, Jeddah 21589, Saudi Arabia*

Correspondence should be addressed to Khalid A. Khan; kzsakhan@hotmail.com

Received 27 May 2013; Accepted 1 August 2013

Academic Editor: Svetlana Ibric

Several Schiff bases **2** have been prepared by the condensation of various methyl ketones **1** with 1,2-ethylenediamine. Subsequent acylation of these Schiff bases with trifluoroacetic anhydride in THF yields the corresponding trifluoroacetyl derivatives **3**. However, *o*-hydroxy- and *o*-methoxy-substituted derivatives yielded substituted-trifluoromethylchroman-4-ones. Additionally, Schiff bases when reacted with acetic anhydride afforded the C-acetylated derivatives **4**. The structures of the isolated compounds were fully determined by spectral methods. Preliminary biological screening of the prepared compounds revealed significant cytotoxic and antimicrobial activities.

1. Introduction

Schiff bases and their complexes have been studied for their interesting and important properties, for example, their ability to reversibly bind with oxygen [1], their catalytic activity in hydrogenation of olefins [2], their photochromic properties [3, 4], and their complexing ability towards some toxic metals [5]. The high affinity for the chelation of the Schiff bases towards the transition metal ions is utilized in preparing their solid complexes.

Recently there has been a considerable interest in the chemistry of hydrazine and hydrazone compounds because of their potential pharmacological applications [6, 7]. They also form basic units in certain dyes [8]. The presence of azomethine and sulfonamide functional group is responsible for antimicrobial activity, which can be altered depending upon the type of substituent present on the aromatic rings [9]. In azomethine derivatives, the C=N linkage is essential for biological activity owing to their excellent chelating properties. Several azomethines were reported to possess remarkable antibacterial, antifungal, anticancer, and diuretic activities [10, 11].

Fluorine plays a significant role in altering the physicochemical characteristics and thereby influencing the electronic, lipophilic, and steric parameters of the organic molecules. Such modulation can profoundly affect the physical and pharmacokinetic properties of drug molecules. Schiff's base of ethylenediamine with 2-furaldehyde and thiophenecarboxaldehyde has been reported [12] but its trifluoroacetyl analogs have not been prepared yet. Trifluoroacetyl groups have been recognized as general binding elements for biological targets with polar active sites. It has also been shown that selective organofluorine interactions with protein residues can be used to substantially enhance protein-ligand binding affinity and selectivity [13]. Compounds containing the trifluoroacetyl group were found to show tumor specific cytotoxic [14] and antimicrobial [15] activities. However, these residues are fully hydrated in aqueous solution, and these hydrates, as part of biomimetic ligands, are good entities to bind to the active sites [16]. In an extension

of our previous study, in the area of synthesis of drugs, semidrugs, and bioactive compounds [17–19], we report the synthesis, characterization, and bacterial growth inhibitory and anticancer activities of trifluoroacetyl derivatives of the Schiff bases of ethylenediamine. An attempt has also been made to compare the antibacterial and anticancer activities of fluorinated and the corresponding nonfluorinated analogs.

2. Experimental

2.1. Chemistry. Melting points were determined in open glass capillaries, on a Gallenkamp melting point apparatus, and were uncorrected. The infrared (IR) spectra were recorded on Perkin-Elmer 297 infrared spectrophotometer, using the NaCl plate technique. Elemental analyses were performed at the Microanalytical Unit, Faculty of Science, King Abdul-Aziz University, Jeddah, Saudi Arabia, and the found values were within ±0.4% of the theoretical values (Table 1). The ^{1}H-NMR spectra were recorded on a Varian EM 360 spectrometer, using tetramethylsilane as the internal standard (Chemical shifts in δ, ppm). Splitting patterns were designated as follows: s: singlet, d: doublet, and m: multiplet (Tables 2 and 3). Followup of the reactions and checking the homogeneity of the compounds were made by TLC on silica gel-protected aluminium sheets (Type 60 F254, Merck), and the spots were detected by the exposure to UV-lamp at λ 254.

*2.1.1. N,N′-Bis(1-substituted-ethylidene)ethane-1,2-diamine (**2**).* A mixture of 1,2-diaminoethane (0.6 g, 10 mmol) and the appropriate ketone **1** (20 mmol) in dry benzene (50 mL) was refluxed using Dean-Stark trap until no more water was collected (about 2 h). Benzene was then removed under reduced pressure, and the residue was treated with methanol. The solid which separated out was recrystallized from ethanol as needles.

*2.1.2. 1,1,1-Trifluoro-4-substituted-4-[(2-{[4,4,4-trifluoro-3-oxo-1-substituted-but-1-en-1-yl]amino}-ethyl)amino]but-3-en-2-one (**3**).* A mixture of the N,N′-Bis(1-substituted ethylidene)ethane-1,2-diamine (10 mmol) in THF (30 mL) and trifluoroacetic anhydride (25 mmol) was refluxed for 2 h. The solid which separated on cooling was recrystallized from ethanol as needles.

*2.1.3. 4-Substituted 4-[2-(1-Substituted-3-oxo-but-1-enylamino)-ethylamino]-but-3-en-2-one (**4**).* A mixture of the N,N′-Bis[1-substituted-ethylidene]ethane-1,2-diamine (10 mmol) in THF (30 mL) and acetic anhydride (25 mmol) was refluxed for 2 h. The solid which separated on cooling was recrystallized from ethanol as needles.

*2.1.4. 2-Substituted 2-Trifluoromethylchroman-4-one (**5**).* A mixture of the N,N′-Bis(1-substituted-ethylidene)ethane-1,2-diamine **2c** and **2d** (10 mmol) in THF (30 mL) and trifluoroacetic anhydride (25 mmol) was refluxed for 2 h. The solid which separated on cooling was recrystallized from ethanol as needles.

2.2. Biological Evaluation

2.2.1. In Vitro MTT Cytotoxicity Assay. All procedures and screening techniques were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Stanford, ME, USA). Cells were batch cultured for 10 days then seeded at concentration of 10×10^3 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 h under 5% CO_2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added, and cells were incubated either alone (negative control) or with different concentrations of the test compounds to give a final concentration of (100 − 50 − 25 − 12.5 − 6.25 − 3.125 − 1.56 − 0.78 μg/mL). DMSO was employed as a vehicle for the dissolution of the tested compounds and its final concentration on the cells was less than 0.2%. Cells were suspended in RPMI 1640 medium (for HepG2 and HT29 cell lines) and DMEM (for MCF 7 cell line), 1% antibiotic-antimycotic mixture (10,000 IU/mL penicillin potassium, 10,000 μg/mL streptomycin sulfate and 25 μg/mL amphotericin B), and 1% L-glutamine in 96-well flat bottom microplate at 37°C under 5% CO_2.

After 48 h of incubation, the medium was aspirated, and 40 μL of MTT salt (2.5 μg/mL) was added to each well and incubated for further 4 h at 37°C under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200 μL of 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, CA, USA) at 595 nm and a reference wavelength of 620 nm. Doxorubicin is used as a positive control cytotoxic agent. The cytotoxicity assay data can be found in Table 4.

2.2.2. Procedure for Antimicrobial Activity. The preliminary anti-microbial activities of compounds **2**–**5** were measured in a concentration of 50 mg/L by disc diffusion method [20, 21]. The prepared compounds were tested for their antimicrobial activity against two types of bacterium, *Staphylococcus aureus* (ATCC 25923) as an example of Gram positive bacteria and *Escherichia coli* (ATCC 25922) as Gram negative bacteria, and the antifungal activity was tested using the pathogenic yeast strains *Candida albicans* and *Aspergillus niger*. DMSO was used as a solvent control, and the standard drugs used were Ampicillin and Griseofulvin. The disc diffusion method was performed using Muller-Hinton agar (Hi-Media) medium. The inhibition zones were measured in mm at the end of an incubation period of 24 h at 37°C for bacteria and 72 h at 24°C for fungi. The zone of inhibition in mm is expressed in Table 5.

2.2.3. Procedure for Antimicrobial Activity Using UV (366 nm) Light. This test was performed as mentioned before but the Petri dishes containing microorganisms and the impregnated disks were exposed to UV light (366) for 3 h before incubation. The obtained data is shown in Table 6.

Table 1: Physical and analytical data of compounds **2–5**.

Compd.	R	Yield (%)	m.p. (°C)	Mol. formula	Calc. %			Found %		
					C	H	N	C	H	N
2a	C_6H_5	80	95–98	$C_{18}H_{20}N_2$	81.78	7.63	10.60	81.80	7.61	10.62
2b	4-BrC_6H_4	86	192–194	$C_{18}H_{18}Br_2N_2$	51.21	4.30	6.64	51.22	4.38	6.60
2c	2-HOC_6H_4	92	190–192	$C_{18}H_{20}O_2N_2$	72.95	6.80	9.45	73.04	6.88	9.48
2d	2-CH_3OC_6H	76	90–92	$C_{20}H_{24}N_2O_2$	74.04	7.46	8.64	74.12	7.50	8.62
2e	2-Thienyl	84	142-143	$C_{14}H_{16}N_2S_2$	60.83	5.83	10.13	60.90	5.91	10.21
2f	3-Thienyl	82	132-133	$C_{14}H_{16}N_2S_2$	60.83	5.83	10.13	60.88	5.87	10.19
2g	2-Furyl	74	113–115	$C_{14}H_{16}N_2O_2$	68.83	6.60	11.47	68.85	6.62	11.40
2h	2-Pyridyl	72	98–100	$C_{16}H_{18}N_4$	72.15	6.81	21.04	72.21	6.73	21.12
2i	4-$CH_3C_6H_4$	88	110–112	$C_{20}H_{24}N_2$	82.15	8.27	9.58	82.06	8.32	9.61
2j	4-$CH_3OC_6H_4$	86	148–150	$C_{20}H_{24}N_2O_2$	74.04	7.46	8.64	74.12	7.51	8.77
2k	3,4-$(CH_3O)_2C_6H_4$	82	108–110	$C_{22}H_{28}N_2O_4$	68.73	7.34	7.29	68.81	7.48	7.33
3a	C_6H_5	72	252–254	$C_{22}H_{18}F_6N_2O_2$	57.90	3.98	6.14	58.03	4.02	6.19
3b	4-BrC_6H_4	76	180–182	$C_{22}H_{16}Br_2F_6N_2O_2$	43.02	2.63	4.56	43.11	2.64	4.60
3c	2-Thienyl	74	158-159	$C_{18}H_{14}F_6N_2O_2S_2$	46.15	3.01	5.98	46.18	3.12	5.82
3d	3-Thienyl	72	163–165	$C_{18}H_{14}F_6N_2O_2S_2$	46.15	3.01	5.98	46.14	3.04	6.01
3e	2-Furyl	69	180–182	$C_{18}H_{14}F_6N_2O_4$	49.55	3.23	6.42	49.65	3.28	6.47
3f	2-Pyridyl	67	196–198	$C_{20}H_{16}F_6N_4O_2$	52.41	3.52	12.22	52.52	3.61	12.18
3g	4-$CH_3C_6H_4$	78	188–190	$C_{24}H_{22}F_6N_2O_2$	59.50	4.58	5.78	59.55	4.56	5.81
3h	4-$CH_3OC_6H_4$	74	196–198	$C_{24}H_{22}F_6N_2O_4$	55.82	4.29	5.42	55.90	4.38	5.60
3i	3,4-$(CH_3O)_2C_6H_4$	70	205–208	$C_{26}H_{26}F_6N_2O_6$	54.17	4.55	19.77	54.28	4.60	19.91
4a	C_6H_5	76	150–152	$C_{22}H_{24}N_2O_2$	75.83	6.94	8.04	75.94	7.10	8.13
4b	4-BrC_6H_4	78	169–171	$C_{22}H_{22}Br_2N_2O_2$	52.20	4.38	5.53	52.28	4.42	5.58
4c	2-Furyl	58	184–186	$C_{18}H_{20}N_2O_4$	65.84	6.14	8.53	65.93	6.22	8.46
5a	2-OHC_6H_4	69	204–206	$C_{10}H_7F_3O_3$	51.74	3.04	—	51.82	3.12	—
5b	2-$CH_3OC_6H_4$	66	105–107	$C_{11}H_9F_3O_3$	53.67	3.68	—	53.77	3.70	—

Table 2: ^{1}H and ^{13}C NMR spectral data (δ/ppm)[a] of compound series **2**.

Compd.	R	^{1}H NMR			^{13}C NMR			
		CH_3 (s)	CH_2 (s)[b]	Ar-H (m)	CH_3	CH_2	C=N	Ar-C
2a	C_6H_5	2.32	3.94	7.62–7.29 (10H)	14.6	48.2	164.8	128.5, 129.1, 130.6, 137.3
2b	4-BrC_6H_4	2.29	3.91	7.46–7.51 (8H)	14.5	48.4	164.6	125.3, 131.3, 132.0, 136.5
2c	2-OHC_6H_4	2.36	3.82	6.77–7.51 (8H)[c]	14.9	48.1	164.5	115.9, 121.2, 124.6, 130.6, 132.3, 157.8
2d	2-CH_3OC_6H	2.38, 3.73[d]	3.79	6.80–7.51 (8H)	14.8, 56.0[d]	47.9	164.2	114.2, 120.9, 122.8, 130.1, 131.8, 162.5
2e	2-Thienyl	2.30	3.50	6.98–7.32 (6H)	15.3	48.3	164.7	126.4, 127.6
2f	3-Thienyl	2.32	3.48	7.02–7.28 (6H)	15.2	48.2	164.8	126.8, 127.9, 130.2
2g	2-Furyl	2.39	3.39	6.51–7.43 (6H)	15.4	45.3	164.6	110.4, 143.6
2h	2-Pyridyl	2.19	3.19	7.63–8.83 (8H)	14.6	48.4	164.4	124.3, 125.7, 135.1, 149.8, 158.6
2i	4-$CH_3C_6H_4$	2.33, 2.41	3.89	7.09–7.51 (8H)	14.6, 20.7	48.3	164.6	128.9, 129.3, 134.3, 140.0
2j	4-$CH_3OC_6H_4$	2.30, 3.75[d]	3.92	6.81–7.54 (8H)	14.7, 56.0[d]	48.0	164.6	114.4, 129.6, 130.3, 163.9
2k	3,4-$(CH_3O)_2C_6H_4$	2.36, 3.73[d] 3.80[d]	3.91	6.69–7.02 (6H)	14.4, 56.3[d], 56.4[d]	48.2	164.8	115.4, 115.8, 122.6, 130.8, 147.8, 150.1

[a]Solution in $CDCl_3$; [b]($2CH_2$, 4H); [c]12.3 (broad singlet, OH); [d]OCH_3.

3. Results and Discussion

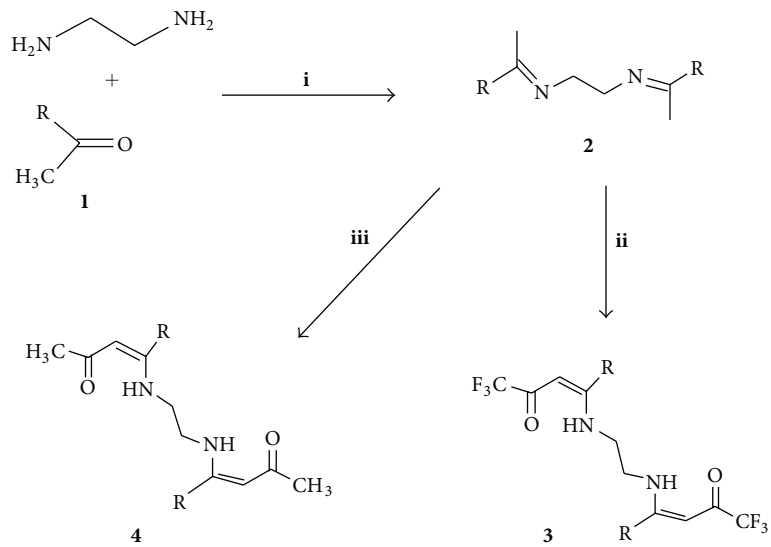
3.1. Chemistry. The synthesis of Schiff bases, reaction involving amine and carbonyl compound, and their metal complexes are well known [22–26]. In general, 1,2-ethylenediamine was reacted with various methyl aryl ketones to give the Schiff bases **2a–k** [27] in moderate to good yields (Scheme 1).

The IR spectra showed a characteristic C=N absorption bands at 1618–1622 cm^{-1}. Their ^{1}H NMR spectra exhibited, beside the aromatic and methyl protons, a singlet of four-proton intensity at δ 2.19–3.94 for the $2CH_2$ (Table 2). Acylation of Schiff bases **2** with trifluoroacetic anhydride in THF yielded the corresponding trifluoroacetyl derivatives **3a–i**. The IR spectra exhibited, beside the absorption bands

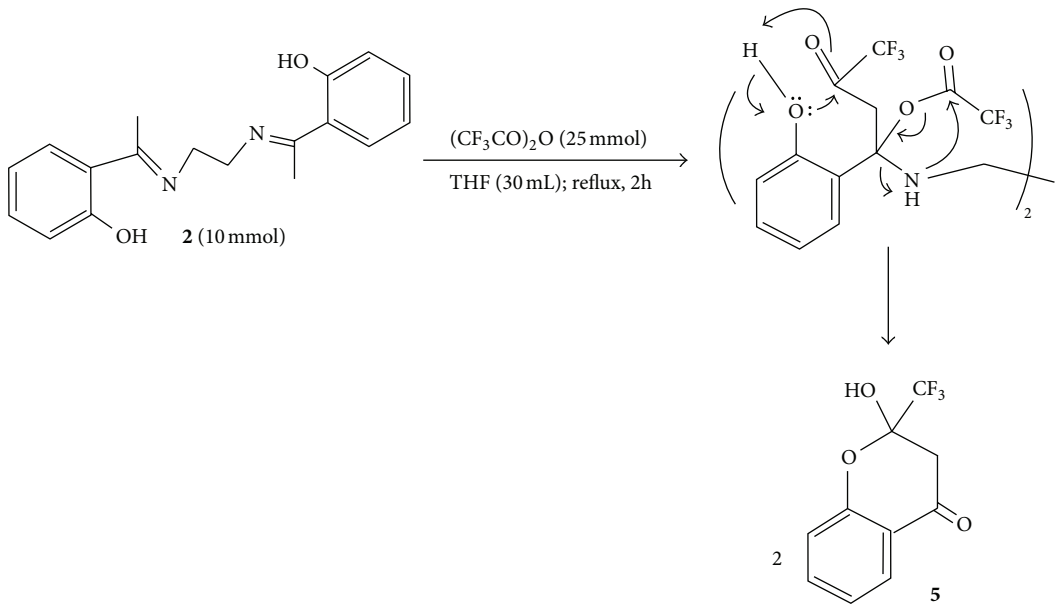
Table 3: ^{1}H and ^{13}C NMR spectral data (δ/ppm)[a] of compounds **3**–**5**.

Compd.	R	^{1}H NMR				^{13}C NMR				
		CH= (s)	CH_2 (s)[b]	Ar-H (m)	Others NH or OH	CH_2	CH= & =C–NH	CF_3	Ar-C	C=O
3a	C_6H_5	5.50	2.91	7.14–7.32 (10H)	10.91	48.9	99.6, 160.8	132.4	126.2, 127.6, 128.3, 134.8	195.8
3b	4-BrC_6H_4	5.46	2.83	7.19–7.42 (8H)	11.02	48.7	98.8, 161.4	132.1	122.3, 128.5, 131.6, 134.2	196.0
3c	2-Thienyl	5.46	2.90	7.31–7.68 (6H)	10.89	48.3	104.5, 161.5	132.2	126.2, 127.4, 130.3, 137.0	196.5
3d	3-Thienyl	5.44	2.92	7.01–7.42 (6H)	10.93	48.5	104.5, 161.3	132.1	120.2, 125.8, 126.1, 135.0	196.4
3e	2-Furyl	5.42	2.94	6.70–7.86 (6H)	10.95	46.2	104.6, 161.3	131.6	111.6, 112.5, 145.2, 155.1	196.3
3f	2-Pyridyl	6.08	2.91	6.50–8.66 (8H)	11.10	48.9	104.3, 165.0	132.2	121.3, 122.2, 149,6, 155.7	196.5
3g	4-$CH_3C_6H_4$	5.48 2.35[c]	2.93	7.01–7.20 (8H)	10.88	48.8, 20.9[c]	98.9, 161.3	132.1	126.1, 129.3, 131.1, 136.6	196.3
3h	4-$CH_3OC_6H_4$	5.51 3.73[d]	2.90	6.72–7.19 (8H)	11.08	48.6 56.0[d]	96.1, 160.8	132.0	114.0, 127.3, 127.6, 161.2	196.5
3i	3,4-$(CH_3O)_2C_6H_4$	5.62	2.90	6.61–6.75 (6H)	11.12	48.9, 56.1[d] 56.3[d]	98.6, 161.5	132.1	112.8, 115.0, 119.5, 128.2, 146.8, 147.6	196.4
4a	C_6H_5	5.46	2.92 2.30[c]	7.14–7.33 (8H)	10.82	48.7	98.6, 161.3	24.8[c]	126.4, 127.7, 128.2, 134.8	196.7
4b	4-BrC_6H_4	5.44	2.90 2.31[c]	7.19–7.39 (8H)	10.76	48.8	98.7, 161.6	24.9[c]	122.2, 128.5, 131.6, 134.0	196.4
4c	2-Furyl	5.38	2.88 2.34[c]	6.68–7.72 (6H)	10.82	46.7	102.3, 160.2	24.5[c]	112.0, 112.9, 144.2, 156.3	195.9
5a	2-OHC_6H_4		3.86[e]	6.85–7.78 (4H)	4.02	35.1	105.0[f]	123.4	114.1, 120.0, 123.1, 129.2, 133.5, 158.9	197.6
5b	2-$CH_3OC_6H_4$		3.91[e] 3.42[d]	6.90–7.82 (4H)		32.6, 45.2[d]	102.3[f]	125.6	114.3, 121.3, 124.0, 130.1, 132.8, 156.4	198.2

[a]Solution in $CDCl_3$; [b]($2CH_2$, 4H); [c]CH_3; [d]OCH_3; [e]H-3 (m, 2H); [f](C-2).



Scheme 1: Synthesis of Schiff bases of 1,2-diamine. **i**: appropriate ketone (20 mmol) 1,2-diaminoethane (10 mmol, 0.6 g), dry benzene (50 mL), and reflux using Dean-Stark trap ~2 h; **ii**: N,N′-bis(1-substituted-ethylidene)-ethane-1,2-diamine (10 mmol), THF (30 mL), $(CF_3CO)O$ (25 mmol), and reflux 2 h; **iii**: N,N′-bis(1-substituted-ethylidene)ethane-1,2-diamine (10 mmol), THF (30 mL), $(CF_3CO)O$ (25 mmol), and reflux 2 h.



Scheme 2: Cyclization of Schiff bases to trifluoromethyl-chromanone.

Table 4: Cytotoxic effects (LC_{50}; μg/mL)[a] of the active compounds (**2**–**5**) on some human tumor cell lines using the MTT assay.

Compound no.	Human colon carcinoma HT29	Human hepatocellular carcinoma HepG2	Human breast cancer MCF 7
2b	41.2	39.4	—
2e	35.8	47.3	51.6
2g	74.3	—[b]	—
2h	61.4	47.2	—
2j	40.3	—	54.2
3a	14.6	25.8	32.4
3b	9.6	23.5	3.2
3c	12.9	31.8	9.7
3e	19.1	26.3	34.2
3f	8.4	20.7	2.1
3g	17.9	29.4	28.2
3h	8.8	21.6	6.4
3i	11.8	25.2	30.1
4b	55.1	48.7	64.9
5a	19.8	37.2	46.4
5b	20.5	28.1	48.9
Doxorubicin[c]	21.1	1.69	2.14

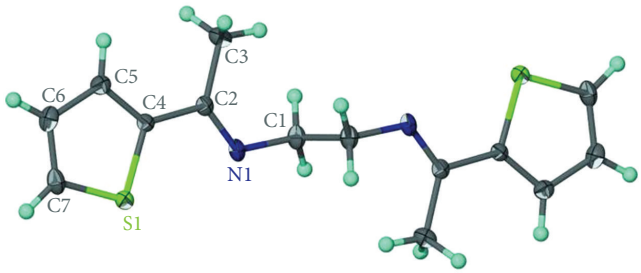
[a]LC_{50}: lethal concentration of the compound which causes death of 50% of the cells in 24 h (μg/mL).
[b]Totally inactive against this cell line. [c]Positive control cytotoxic agent.

corresponding to the NH group at 3216–3288 cm^{-1}, a characteristic carbonyl band at 1672–1675 cm^{-1}.

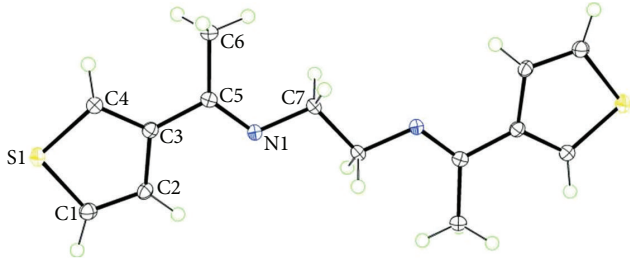
Their ^{1}H NMR spectra displayed signals due to aromatic and aliphatic protons but lacked signals characteristic of methyl group of the corresponding Schiff bases (Table 3). The structures of the Schiff bases **2** and the trifluoroacetyl derivatives **3** were further confirmed from their ^{13}C NMR spectra (Table 3) as well as their X-ray crystallography [28–30] (Figure 1). However, reaction of the Schiff bases **2** with acetic anhydride afforded the corresponding acetyl derivatives **4**. The IR of **4a–c** showed a carbonyl band at 1664–1666 cm^{-1} beside the absorption bands of the NH group at 3335–3372 cm^{-1}. Their ^{1}H NMR spectra exhibited, beside the aromatic and aliphatic protons, a singlet at δ 2.30-2.31 for the CH_3 group (Table 2). The structures were further confirmed from their ^{13}CNMR spectra data (Table 3). Moreover, reaction of the Schiff bases **2c** and **2d** with trifluoroacetic anhydride in THF afforded the corresponding trifluoromethylchroman-4-one derivatives **5a** and **5b** instead of the expected trifluoroacetyl derivatives. A possible mechanism for the reaction is shown in Scheme 2. The IR spectra of chromanone derivatives **5** exhibited a strong carbonyl band at 1668–1670 cm^{-1}, while their ^{1}H NMR spectra displayed beside the aromatic protons a singlet of two-proton intensity at δ 3.86–3.91 for H-3 (Table 3). The structures of **5** were further confirmed from their ^{13}CNMR spectra as well as X-ray crystallography [31] (Figure 1).

3.2. Biological Evaluation

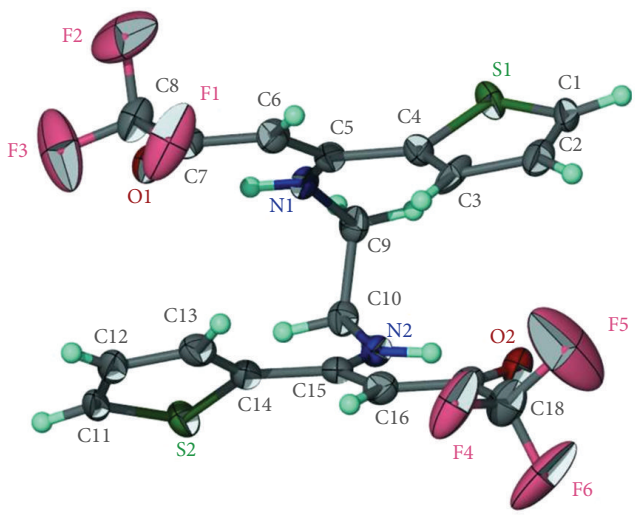
3.2.1. In Vitro MTT Cytotoxicity Assay. Twenty-one analogs **2a–h**, **2j**, **3a–c**, **3e–i**, **4a**,**b**, and **5a**, **5b** were selected to be evaluated for their *in vitro* cytotoxic effect via the standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) method [32, 33] against a panel of three human tumor cell lines, namely, Caucasian breast adenocarcinoma MCF7, hepatocellular carcinoma HepG2, and colon carcinoma HT29. The results are presented in Table 4 as LC_{50}



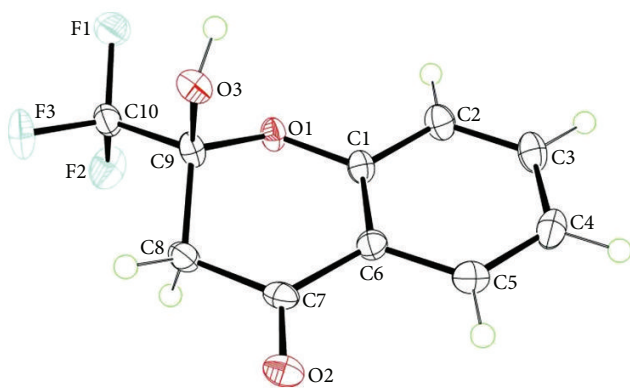
(a) N,N′-Bis[1-(thiophen-2-yl)ethylidene]ethane-1,2-diamine (**2e**)



(b) N,N′-Bis[(E)-1-(thiophen-3-yl)ethylidene]ethane-1,2-diamine (**2f**)



(c) 1,1,1-Trifluoro-4-(thiophen-2-yl)-4-[(2-{[4,4,4-trifluoro-3-oxo-1-(thiophen-2-yl)but-1-en-1-yl]amino}ethyl)amino]but-3-en-2-one (**3c**)



(d) 2-Hydroxy-2-trifluoro-methyl-3,4-dihydro-2H-1-benzopyran-4-one (**5a**)

Figure 1: X-ray crystal structures of **2e**, **2f**, **3c**, and **5a**.

Table 5: Antibacterial and antifungal data of Schiff bases and their acyl derivatives **2**–**5**.

Compound	Zone of inhibition in mm			
	Escherichia coli	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*
2a	12	14	13	12
2b	17	17	15	15
2c	15	15	14	16
2d	14	16	13	15
2e	15	17	16	14
2f	16	15	14	15
2g	18	16	15	14
2h	19	18	18	17
2i	13	15	14	12
2j	15	14	16	11
2k	16	13	14	10
3a	24	25	26	25
3b	31	29	28	29
3c	27	26	26	26
3d	28	27	28	25
3e	27	25	26	27
3f	30	28	29	28
3g	27	26	25	24
3h	29	27	26	25
3i	28	24	25	26
4a	19	16	20	18
4b	22	20	21	20
4c	17	16	15	16
5a	26	25	24	22
5b	27	24	25	21
Ampicillin	32	28	—	—
Griseofulvin	—	—	31	29

(μg/mL) which is the lethal concentration of the compound which causes death of 50% of the cells in 24 h.

The obtained data revealed that the three tested human tumor cell lines exhibited variable degree of sensitivity profiles towards sixteen of the tested compounds **2b**, **2e**, **2g**, **2h**, **2j**, **3a**–**c**, **3e**–**i**, **4b**, **5a**, and **5b**. Among these, the human colon carcinoma HT29 cell line showed pronounced sensitivity against fluorinated compounds **3b**, **3f**, and **3h** with LC_{50} values of 9.6, 8.4, and 8.8 μg/mL, respectively. In addition, a significant cytotoxic activity was displayed by compounds **3c** and **3i** against the same cell line (12.9 and 11.8 μg/mL). Meanwhile, compounds **3e**, **3 g**, **5a** and **5b** revealed moderate cytotoxicity profile against colon carcinoma HT29 with LC_{50} values 19.7, 17.9, 19.8 and 20.5 μg/mL, respectively. Nonfluorinated compounds **2b**, **2e**, **2h**, and **4b** were able to exhibit mild activity against the same cell line with LC_{50} values range of 35.8–61.4 μg/mL. Low activity was displayed by compounds **2g** at LC_{50} value 74.3 μg/mL. However, the growth of the human hepatocellular carcinoma HepG2 cell

Table 6: Antibacterial and antifungal data of Schiff bases and their acyl derivatives (**2**–**5**) using UV (366) light.

Compound	Zone of inhibition in mm Antibacterial activity		Zone of inhibition in mm Antifungal activity	
	Escherichia coli	*Staphylococcus aureus*	*Aspergillus niger*	*Candida albicans*
2a	13	—	14	13
2b	18	—	—	16
2c	—	17	—	—
2d	15	—	—	16
2e	16	—	—	16
2f	17	16	15	16
2g	19	17	17	15
2h	20	—	—	—
2i	14	16	—	—
2j	—	—	17	13
2k	—	14	—	—
3a	25	26	—	26
3b	—	—	—	—
3c	—	—	27	27
3d	29	28	29	—
3e	28	—	—	28
3f	—	—	—	—
3g	28	27	—	—
3h	30	28	26	—
3i	—	26	—	—
4a	20	17	21	19
4b	—	21	—	21
4c	—	17	—	—
5a	27	26	—	—
5b	—	25	—	—
Ampicillin	32	28	—	—
Griseofulvin	—	—	31	29

line was found to be moderately inhibited by fourteen of the active compounds **2b**, **2e**, **2j**, **3a**, **3b**, **3c**, **3e**, **3f–i**, **4b**, **5a**, and **5b** with LC_{50} values range of 20.7–48.7 μg/mL. Among these, the highest cytotoxic activity was displayed by fluoro analogs **3b, 3e, 3f**, **3h**, and **3i** which were almost equally potent (LC_{50} values 23.5, 26.3, 20.7, 21.6, and 25.2 μg/mL, resp.). On the other hand, human breast cancer MCF 7 was proved to be the least sensitive among the cell lines tested as it was affected by only seven of the test compounds. However, an outstanding growth inhibition potential was shown by compounds **3b**, **3c**, **3f** and **3h** as evidenced from their LC_{50} values (3.2, 9.7, 2.1, and 6.4 μg/mL, resp.). The remaining eight active compounds **2d**, **3a**, **3e**, **3g**, **3i**, **4b**, **5a**, and **5b** showed mild to moderate activity against the same cell line with LC_{50} values range of 28.2–64.9 μg/mL (Table 4). Further interpretation of the results revealed that, compounds **3b**, **3c**, **3e** and **3f**, and **5a** showed considerable broad spectrum of cytotoxic activity against the three tested human tumor cell lines. In particular, compound **3f** proved to be the most active member in this study with a broad spectrum of activity against all the tested cell lines, with special effectiveness against the human colon carcinoma HT29 and human breast cancer MCF 7 cell lines (LC_{50} values 8.4 and 2.1 μg/mL, resp.) (Table 4). A close examination of the structure of the active compounds showed that the presence of trifluoroacetyl moiety with 2-pyridyl nucleus in diamine skeleton is the most favorable substituent when compared with the nonfluorinated analogs. The presence of trifluoroacetyl group in the diamine skeleton (as in compounds **3** and **5**) is responsible for the high activity displayed by these analogs. The Schiff bases without the trifluoroacetyl moiety resulted in moderate to weakly active compounds (**2** and **4b**), whereas other compounds are completely inactive.

3.2.2. Antimicrobial Activity. Compounds **2**–**5** were screened *in vitro* for their antimicrobial and antifungal activities against *Escherichia coli, Staphylococcus aureus, Aspergillus niger,* and *Candida albicans*. The zones of inhibition formed for the compounds against bacteria and fungi are summarized in Table 5. The overall results evidently suggest that

compounds containing trifluoroacetyl substituent exhibited relatively better antimicrobial and antifungal activities than all the nonfluorinated analogs indicating a positive role of fluorine substitution in the present series. Compounds **3** and **5** were, however, significantly active when compared with rest of the series. Furthermore, after using UV-Visible light, most of the tested compounds showed an additional activity especially towards *Escherichia coli* and *Candida albicans* (Table 6). All test data in Tables 5 and 6 were of average values from triplicate runs, and the test compounds showed reduced antimicrobial activities when compared with their respective standards.

4. Conclusions

In this paper, several new N,N′-bis(1-substituted ethylidene)ethane-1,2-diamines were synthesized by the condensation of 1,2-ethylenediamine with the appropriate methyl ketone. Acetylating these Schiff bases with trifluoroacetic anhydride or acetic anhydride afforded the corresponding acetyl derivatives. Structures of the prepared compound were confirmed by elemental analysis, IR, ^{1}H, ^{13}C NMR spectral analysis, and X-ray crystallography. Preliminary biological testing of these compounds revealed that trifluoroacetyl derivatives exhibited significant cytotoxic and antimicrobial activities. Further, the incorporation of trifluoroacetyl group is justified by a comparative study with the nonfluorinated analogs. The fluorinated analogs were found to be more active than their nonfluorinated counterparts.

Acknowledgments

The authors are deeply thankful to the authorities of the Bioassay-Cell Culture Laboratory, Drug Discovery Unit, National Research Centre, Cairo, Egypt, for their efforts in performing the MTT cytotoxicity assay. The authors would like to thank Ms. Samina Shamim, English Language Institute, King Abdulaziz University, for reviewing the language and grammar of the paper.

References

[1] T. Suzuki, Y. Suzuki, K. Hachisu, and H. Shinozaki, "Photoreversible oxygen binding of a cobalt schiff-base complex with an axially coordinating photo-isomerizable polymer ligand," *Polymers for Advanced Technologies*, vol. 22, no. 8, pp. 1278–1282, 2011.

[2] S. Alexander, V. Udayakumar, and V. Gayathri, "Hydrogenation of olefins by polymer-bound palladium(II) Schiff base catalyst," *Journal of Molecular Catalysis A*, vol. 314, no. 1-2, pp. 21–27, 2009.

[3] E. Hadjoudis, A. Rontoyianni, K. Ambroziak, T. Dziembowska, and I. M. Mavridis, "Photochromism and thermochromism of solid *trans-N,N*-bis-(salicylidene)-1,2-cyclohexanediamines and *trans-N,N*-bis-(2-hydroxy-naphylidene)-1,2-cyclohexanediamine," *Journal of Photochemistry and Photobiology A*, vol. 162, no. 2-3, pp. 521–530, 2004.

[4] Y. Chen and D. X. Zeng, "Study on photochromic diarylethene with phenolic schiff base: preparation and photochromism of diarylethene with benzoxazole," *Journal of Organic Chemistry*, vol. 69, no. 15, pp. 5037–5040, 2004.

[5] O. Kocyigit, A. N. Kursunlu, and E. Guler, "Complexation properties and synthesis of a novel Schiff base with triphenylene nucleus," *Journal of Hazardous Materials*, vol. 183, no. 1-3, pp. 334–340, 2010.

[6] X. Ran, L. Wang, D. Cao, Y. Lin, and J. Hao, "Synthesis, characterization and in vitro biological activity of cobalt(II), copper(II) and zinc(II) Schiff base complexes derived from salicylaldehyde and D,L-selenomethionine," *Applied Organometallic Chemistry*, vol. 25, no. 1, pp. 9–15, 2011.

[7] A. Prakash and D. Adhikari, "Application of Schiff bases and their metal complexes: a review," *International Journal of ChemTech Research*, vol. 3, no. 4, pp. 1891–1896, 2011.

[8] H. Karaer and I. E. Gümrükçüoğlu, "Synthesis and spectral characterisation of novel azo-azomethine dyes," *Turkish Journal of Chemistry*, vol. 23, no. 1, pp. 67–71, 1999.

[9] S. Kumar, M. S. Niranjan, K. C. Chaluvaraju, C. M. Jamakhandi, and D. Kadadevar, "Synthesis and antimicrobial study of some Schiff bases of sulfonamides," *Journal of Current Pharmaceutical Research*, vol. 1, no. 1, pp. 39–42, 2010.

[10] C. T. Supuran, M. Barboiu, C. Luca, E. Pop, M. E. Brewster, and A. Dinculescu, "Carbonic anhydrase activators. Part 14. Syntheses of mono and bis pyridinium salt derivatives of 2-amino-5-(2-aminoethyl)- and 2-amino-5-(3-aminopropyl)-1,3,4-thiadiazole and their interaction with isozyme II," *European Journal of Medicinal Chemistry*, vol. 31, no. 7-8, pp. 597–606, 1996.

[11] A. M. Asiri and S. A. Khan, "Synthesis and anti-bacterial activities of some novel schiff bases derived from aminophenazone," *Molecules*, vol. 15, no. 10, pp. 6850–6858, 2010.

[12] C. A. Bolos, G. S. Nikolov, L. Ekateriniadou, A. Kortsaris, and D. A. Kyriakidis, "Structure-activity relationships for some diamine, triamine and Schiff base derivatives and their copper(II) complexes," *Metal-Based Drugs*, vol. 5, no. 6, pp. 323–332, 1998.

[13] J. A. Olsen, D. W. Banner, P. Seiler et al., "Fluorine interactions at the thrombin active site: protein backbone fragments H–Cα–C=O comprise a favorable C-F environment and interactions of C–F with electrophiles," *ChemBioChem*, vol. 5, no. 5, pp. 666–675, 2004.

[14] H. Hatano, F. Takekawa, K. Hashimoto et al., "Tumor-specific cytotoxic activity of 1,2,3,4-tetrahydroisoquinoline derivatives against human oral squamous cell carcinoma cell lines," *Anticancer Research*, vol. 29, no. 8, pp. 3079–3086, 2009.

[15] A. A. Bekhit, H. M. Abdel-Rahman, and A. A. Guemei, "Synthesis and biological evaluation of some hydroxypyrazole derivatives as anti-inflammatory-antimicrobial agents," *Archiv der Pharmazie*, vol. 339, no. 2, pp. 81–87, 2006.

[16] C. Mattos, C. R. Bellamacina, E. Peisach et al., "Multiple solvent crystal structures: probing binding sites, plasticity and hydration," *Journal of Molecular Biology*, vol. 357, no. 5, pp. 1471–1482, 2006.

[17] H. M. Faidallah, K. A. Khan, and A. M. Asiri, "Synthesis and biological evaluation of new 3- trifluoromethylpyrazolesulfonylurea and thiourea derivatives as antidiabetic and antimicrobial agents," *Journal of Fluorine Chemistry*, vol. 132, no. 2, pp. 131–137, 2011.

[18] H. M. Faidallah, K. A. Khan, and A. M. Asiri, "Synthesis and biological evaluation of new 3,5-di(trifluoromethyl)-1,2,4-triazolesulfonylurea and thiourea derivatives as antidiabetic

and antimicrobial agents," *Journal of Fluorine Chemistry*, vol. 132, no. 11, pp. 870–877, 2011.

[19] H. M. Faidallah and K. A. Khan, "Synthesis and biological evaluation of new barbituric and thiobarbituric acid fluoro analogs of benzenesulfonamides as antidiabetic and antibacterial agents," *Journal of Fluorine Chemistry*, vol. 142, pp. 96–104, 2012.

[20] W. R. Kirkpatrick, T. M. Turner, A. W. Fothergill et al., "Fluconazole disk diffusion susceptibility testing of *Candida* species," *Journal of Clinical Microbiology*, vol. 36, no. 11, pp. 3429–3432, 1998.

[21] T. Premkumar and S. Govindarajan, "Antimicrobial study of pyrazine, pyrazole and imidazole carboxylic acids and their hydrazinium salts," *World Journal of Microbiology and Biotechnology*, vol. 21, no. 4, pp. 479–480, 2005.

[22] M. R. Bermejo, M. I. Fernández, E. Gómez-Fórneas et al., "Self-assembly of dimeric MnIII-Schiff-base complexes tuned by perchlorate anions," *European Journal of Inorganic Chemistry*, no. 24, pp. 3789–3797, 2007.

[23] A. Pui, C. Dobrota, and J.-P. Mahy, "Electrochemical and spectroscopic characterization and catalytic activity of Co(II) complexes of tetra-chloro-R-Salen ([tClSalen=bis(3,5-di-chloro- α-R salicylidene)ethylenediamine]) and tetra-chloro-R-Salophen ([tClSalophen=bis(3,5-di-chloro-α-R salicylidene)-1,2-phenylenediamine]), R=H, CH_3, CH_2–CH_3," *Journal of Coordination Chemistry*, vol. 60, no. 5, pp. 581–595, 2007.

[24] S. R. Doctrow, K. Huffman, C. B. Marcus et al., "Salen-manganese complexes as catalytic scavengers of hydrogen peroxide and cytoprotective agents: structure-activity relationship studies," *Journal of Medicinal Chemistry*, vol. 45, no. 20, pp. 4549–4558, 2002.

[25] C. S. Higham, D. P. Dowling, J. L. Shaw, A. Cetin, C. J. Ziegler, and J. R. Farrell, "Multidentate aminophenol ligands prepared with Mannich condensations," *Tetrahedron Letters*, vol. 47, no. 26, pp. 4419–4423, 2006.

[26] U. Casellato, S. Tamburini, P. Tomasin, and P. A. Vigato, "Cyclic and acyclic compartmental Schiff bases, their reduced analogues and related mononuclear and heterodinuclear complexes," *Inorganica Chimica Acta*, vol. 357, no. 14, pp. 4191–4207, 2004.

[27] B. N. Ghose, "Schiff base complexes of boron: reaction of boron acetate with dibasic quadridentate Schiff bases," *Synthesis and Reactivity in Inorganic and Metal-Organic Chemistry*, vol. 16, no. 10, pp. 1383–1393, 1986.

[28] A. M. Asiri, A. O. Al-Youbi, H. M. Faidallah, K. A. Alamry, and S. W. Ng, "N,N′-Bis[1-(thiophen-2-yl)ethylidene]ethane-1,2-diamine," *Acta Crystallographica Section E*, vol. 67, no. 9, article o2465, 2011.

[29] A. M. Asiri, A. O. Al-Youbi, H. M. Faidallah, and S. W. Ng, "1,1,1-Trifluoro-4-(thiophen-2-yl)-4-[(2-{[4,4,4-trifluoro-3-oxo-1-(thiophen-2-yl)but-1-en-1-yl]amino}ethyl)amino]but-3-en-2-one," *Acta Crystallographica Section E*, vol. 67, no. 10, pp. o2659–o2660, 2011.

[30] A. M. Asiri, H. M. Faidallah, K. A. Khan, S. W. Ng, and E. R. T. Tiekink, "N,N′-Bis[(E)-1-(thio-phen-3-yl)ethyl-idene]ethane-1,2-diamine," *Acta Crystallographica Section E*, vol. 68, no. 4, article o1026, 2012.

[31] A. M. Asiri, H. M. Faidallah, K. A. Alamry, S. W. Ng, and E. R. T. Tiekink, "2-Hydroxy-2-trifluoro-methyl-3, 4-dihydro-2H-1-benzopyran-4-one," *Acta Crystallographica Section E*, vol. E68, no. 7, article o2299, 2012.

[32] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.

[33] F. Denizot and R. Lang, "Rapid colorimetric assay for cell growth and survival—Modifications to the tetrazolium dye procedure giving improved sensitivity and reliability," *Journal of Immunological Methods*, vol. 89, no. 2, pp. 271–277, 1986.