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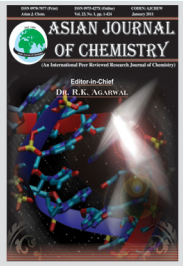

Microwave-Assisted Synthesis of 2,4,5-Triphenyl-1*H*-imidazole Containing Schiff Base Derivatives with Potential Antioxidant and Anticancer Activities

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2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)acetohydrazide (**3**) was utilized as key intermediate for the synthesis of some new Schiff bases **4a-k** and **5** under reflux temperature as well as microwave irradiation. All the synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR and HRMS spectral studies. The antioxidant and anticancer activities of these newly synthesized Schiff base derivatives containing 2,4,5-triphenyl-1*H*-imidazole moiety have been evaluated.

Key Words: Schiff base, 2,4,5-Triphenyl-1*H*-imidazole, Microwave irradiation, Antioxidant activity, Anticancer activity.

INTRODUCTION

Imidazole is an important moiety of the five-membered ring heterocycles which is widely present in naturally occurring molecules[1]. Compounds with imidazole moiety have also been found to possess many pharmacological properties and are widely implicated in biochemical processes[2]. Many of the substituted imidazoles and 2-thioimidazoles present in compounds that have fungicidal[3], herbicidal[4], antiasthmatic[5], antiinflammatory[6], antiulcerative[7] and antithrombotic[8] activities. These types of compounds are also known as plant growth regulators and therapeutic agents[9]. Imidazole compounds are known to possess anti HIV[10], anticonvulsant[10], antisenescence[11], antimuscarinic[12], antiarthritic[13], cardiotonic[14], non-sedative anxiolytic[15] activities, inhibitors of Acyl CoA Cholesterol O-acyl transferase (ACAT)[16], HMG CoA reductase (HMGR)[17], calcium antagonist and inhibitors of thromboxane A2 synthase[18], antihistaminics[19], tranquillizers[20], antiparkinsons[21] and MAO inhibitor[22].

Similarly Schiff bases (>C=N-) form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications[23]. It is well established that the biological activity associated with the hydrazone compounds is attributed to the presence of active pharmacophore (-CONH-N=C-). Hence many hydrazone compounds containing this active moiety showed good anticancer bioactivities[24]. It is, therefore, predicted that imidazoles containing Schiff base would exhibit enhanced antitumor and other biological activities.

Microwave-assisted organic synthesis (MAOS) has become a tremendously growing area in chemistry since 1986[25]. As well as being able to reduce reaction times and increase product yields, chemists are finding that by using microwave heating it is possible to open up new avenues for synthesis. Furthermore, reactions under microwave have the great advantage of using small amounts or no organic solvents, making such reactions more eco-friendly and generate less side products. Compared to traditional methods, this technique is more convenient and easily controllable.

In view of the potentiality of these Schiff base chromopores and the continued interest in the development of imidazole derivatives, the present work has been carried out, to synthesise some new Schiff base derivatives containing 2,4,5-triphenyl-1*H*-imidazole moiety under reflux conditions/microwave irradiation, characterize and explore their biological applications.

EXPERIMENTAL

Infrared (IR) spectra were recorded at room temperature from 4000-400 cm^{-1} with KBr pellets, using Avatar 330 equipped with DTGS detector. The ^{1}H NMR was measured on a Bruker AMX-500 instrument at room temperature using the X-WIN NMR version X-WIN NMR 1.3 cn drx software. The ^{1}H NMR was measured for *ca.* 0.03 M solutions in $CDCl_3$ and DMSO-d_6 using TMS as internal reference. The coupling constants *J* are in Hz. Mass spectra were obtained using Jeol GC Mate II HRMS (EI) and LC-MS-MS (3200 Q-trap). Shimadzu UV-1700(E) 23 OCE model UV-visible spectro-

photometer was used for the determination of absorbance values for all the synthesized compounds, at a wavelength of 517 nm using methanol as a solvent. Melting points were determined in open capillaries and are uncorrected. All reagents were purchased from Aldrich, Hi Media and Qualigens and used without further purification.

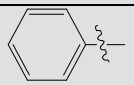
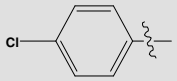
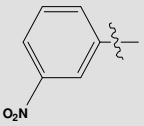
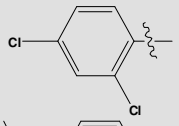
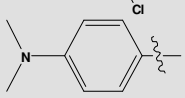
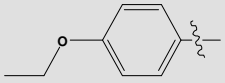
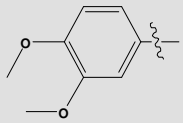
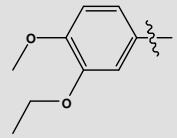
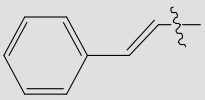
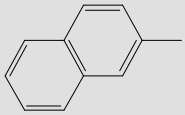
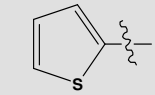
Procedure for the synthesis of ethyl 2-(2,4,5-triphenyl-1*H*-imidazol-1-yl)acetate (2): To a solution of 2,4,5-triphenyl-1*H*-imidazole (**1**) in dry DMF, anhydrous potassium carbonate (1.0 molar equiv) and ethyl chloro acetate (1.0 molar equiv) were added. The resultant mixture was stirred at 75-80 ºC for 52 h, cooled and then the reaction mixture was added to a large amount of water. The solid separated was filtered, washed with excess of water. The crude product was purified by crystallization from ethanol. m.p. 148-150 ºC; 82-84 % yield; IR (KBr, ν_{max}, cm^{-1}): 3056 (Ar C-H), 2977 (C-H), 1742 (C=O), 1602 (C=C), 1204 (C-N), 1027(C-O-C); ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.18 (3H, t, *J* = 7.0 Hz, CH_3), 4.15 (2H, q, *J* = 7.1 Hz, OCH_2), 4.50 (2H, s, N-CH_2-CO), 7.15-7.24 (3H, 2t, *J* = 7.5 Hz, *p*-Ar-H on 2,4,5-phenyl rings), 7.42 (2H, dd, *J* = 3.5 and 2.5 Hz, *o*-Ar-H on 4-phenyl ring), 7.47-7.51 (6H, m, m,m'-Ar-H on 2,4,5-phenyl rings), 7.58 (2H, d, *J* = 7.5 Hz, *o*'-H on 5-phenyl ring), 7.68 (2H, dd, *J* = 2.0 and 1.5 Hz, *o*-Ar-H on 2-phenyl rings); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ_C: 14.04, 46.81, 61.77, 125.58, 126.44, 126.85, 127.46, 127.97, 128.07, 128.51, 128.73, 129.01, 129.13, 129.22, 129.98, 130.55, 130.73, 131.07, 134.29, 137.76, 148.30, 168.36; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{25}H_{22}N_2O_2$: 382.1681; found. (%): 382.1700.

Procedure for the synthesis of 2-(2,4,5-triphenyl-1*H*-imidazol-1-yl)acetohydrazide (3): Ethyl 2-(2,4,5-triphenyl-1*H*-imidazol-1-yl)acetate (**2**) (0.01 mol) in ethanol (20 mL) was stirred at room temperature for 20 min. To this mixture hydrazine hydrate (0.014 mol) was added. The resultant mixture was refluxed for 3 h, cool the reaction mixture and the separated solid filtered with a sintered glass funnel. The residue was washed with ethanol, dried and then desiccated to afford a crystalline powder. The powder was recrystallized from chloroform/methanol. m.p. 236-238 ºC; 90-94 % yield; IR (KBr, ν_{max}, cm^{-1}): 3292 (N-H), 3043 (Ar C-H), 2937 (C-H), 1667 (C=O), 1276 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 4.24 (2H, bs, NH_2), 4.35 (2H, s, N-CH_2-CO), 7.12-7.22 (3H, 2t, *J* = 7.5 Hz, *p*-Ar-H on 2,4,5-phenyl rings), 7.37 (2H, t, *J* = 3.5 Hz, *o*-Ar-H on 4-phenyl ring), 7.42 (2H, d, *J* = 7.5 Hz, *o*-Ar-H on 5-phenyl ring), 7.48-7.51 (6H, m, Ar-H on 2,4,5-phenyl rings), 7.67 (2H, dd, *J* = 8.5 and 7.5 Hz, *o*,*o*'-Ar-H on 2-phenyl ring), 9.08 (1H, s, CONH); ^{13}C NMR (125.75 MHz, DMSO-d_6) δ_C: 46.50, 126.55, 126.75, 128.57, 128.93, 129.08, 129.13, 129.41, 129.50, 129.55, 130.80, 130.87, 130.95, 131.21, 131.28, 134.95, 136.74, 147.96, 167.26; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{23}H_{20}N_4O$: 368.1637; found. (%): 368.1711.

Procedure for the synthesis of 2-(2,4,5-triphenyl-1*H*-imidazol-1-yl)-N'-(substituted aryl)acetohydrazide (4a-k)

Method A: A mixture of compound **3** (0.01 mol) in chloroform/methanol (1:1) mixture (30 mL), aryl/heteroaromatic aldehyde (0.01 mol) and 1 mL of glacial acetic acid was refluxed for a period as shown in Table-1. The progress of reaction was monitored by TLC. After completion of reaction solid product thus obtained was poured in cold water. The separated product was filtered washed with ice cold water and dried at room temperature.

TABLE-1
PHYSICAL DATA OF THE COMPOUNDS **4a-k**

Entry	R	Reaction time[a] (min)	Yield[b] (%)	m.p. (ºC)
4a		60/2	88/94	168-170
4b		90/2	86/93	150-152
4c		120/3	83/88	114-116
4d		120/3	91/96	182-184
4e		180/3	84/92	250-252
4f		120/2	82/89	158-160
4g		90/2.5	87/95	140-142
4h		120/3	81/85	240-242
4i		180/3	83/87	170-172
4j		150/3	84/86	166-168
4k		120/3	81/89	212-214

*At reflux temperature/microwave irradiation. **Isolated yields at reflux temperature/microwave irradiation.

Method B: A mixture of compound **3** (0.01 mol) and hetero aromatic aldehyde (0.01 mol) wetted with glacial acetic acid (2 drops) was ground by pestle in mortar at room temperature followed by the addition of DMSO (2 mL). The resulting reaction mixture was subjected to microwave irradiation using microwave oven (MW domestic type oven 800W SANYO) at 20 % intensity for a period as shown in Table-1 (4 to 6 pulses each of 30 s) and the product was set aside to cool. The progress of reaction was monitored by TLC. After completion of the reaction, reaction mixture was poured into ice-cold water. The separated product was filtered, washed with excess of cold water and dried at room temperature.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(benzylidene)acetohydrazide (4a): IR (KBr, ν_{max}, cm^{-1}): 3220 (N-H), 3064 (Ar C-H), 2932 (C-H), 1674 (C=O), 1559 (C=N), 1240 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 4.39 and 5.02 (2H, 2s, N-CH_2-CO), 7.15-7.24 (3H, 2t, *J* = 7.5 Hz, *p*-Ar-H on 2,4,5-phenyl rings), 7.28-7.37 (3H, m, *m*,*p*,*m*'-Ar-H on benzylidene ring), 7.38-7.44 (6H, m, *p*-Ar-H on 2,4,5-phenyl rings), 7.47 (4H, d, *J* = 6.5 Hz, *o*,*o*'-Ar-H on 4,5-phenyl ring), 7.60 (2H, d, *J* = 7.5 Hz, *o*-Ar-H on benzylidene ring), 7.67 (1H, s, HC=N), 7.76 (2H, d, *J* = 7.0 Hz, *o*,*o*'-Ar-H on 2-phenyl ring), 10.15 (1H, 2s, CONH); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ_C: 46.53, 126.42, 126.99, 127.28, 128.10, 128.72, 128.92, 129.07, 129.15, 130.50, 130.57, 130.69, 130.81, 131.01, 132.95, 134.40, 137.69, 145.56, 148.63, 169.95; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{30}H_{24}N_4O$: 456.1950; found (%): 456.1990.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(4-chlorobenzylidene)acetohydrazide (4b): IR (KBr, ν_{max}, cm^{-1}): 3223 (N-H), 3063 (Ar C-H), 2930 (C-H), 1692 (C=O), 1602 (C=N), 1246 (C-N); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 4.49 and 4.97 (2H, 2s, N-CH_2-CO), 7.14-7.58 (17H, m, Ar-H), 7.73 (2H, d, *J* = 7.4 Hz, *o*-Ar-H on benzylidene ring), 7.96 (1H, dd, *J* = 11.1, 9.1 Hz, HC=N), 9.88 and 9.48 (1H, 2s, CONH); LC-MS (m/z): 491.2 [M + 1], 492.2 [M + 2].

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(3-nitrobenzylidene)acetohydrazide (4c): IR (KBr, ν_{max}, cm^{-1}): 3221 (N-H), 3062 (Ar C-H), 1699 (C=O), 1607 (C=N), 1529 (N=O), 1271 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 4.59 and 5.02 (2H, 2s, N-CH_2-CO), 7.13-7.23 (3H, 2t, *J* = 7.5 Hz, *p*-Ar-H on 2,4,5-phenyl rings), 7.39-7.51 (10H, m, Ar-H), 7.64-7.69 (3H, m, *o*',*m*,*p*-Ar-H on benzylidene ring), 7.99-8.21 (3H, m, *o*-Ar-H on 2-phenyl and benzylidene rings), 8.32 (1H, s, HC=N), 11.82 (1H, s, CONH); HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{30}H_{23}N_5O_3$: 501.1801; found (%): 501.1850.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(2,4-dichlorobenzylidene)acetohydrazide (4d): IR (KBr, ν_{max}, cm^{-1}): 3220 (N-H), 3058 (Ar C-H), 1697 (C=O), 1585 (C=N), 1254 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 4.24 and 4.95 (2H, 2s, N-CH_2-CO), 7.15-7.7.20 (4H, m, *p*- and *m*'-Ar-H on 2,4,5-phenyl and benzylidene rings), 7.29 (1H, dd, *J* = 2, 2.5 Hz, *m*-Ar-H on benzylidene ring), 7.37-7.46 (9H, m, *m,m*'-Ar-H on 2,4,5-phenyl ring, *o,o*'-Ar-H on 4-phenyl ring and *o*'-Ar-H on benzylidene ring), 7.54 (2H, d, *J* = 8.0 Hz, *o*,*o*'-Ar-H on 4-phenyl ring), 7.73 (2H, d, *J* = 7.0 Hz, *o*,*o*'-Ar-H on 2-phenyl ring), 7.98 (1H, m, HC=N), 10.38 (1H, s, CONH); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ_C: 46.44, 126.46, 126.98, 127.42, 127.77, 128.08, 128.70, 128.93, 129.05, 129.09, 129.19, 129.71, 130.40, 130.53, 130.73, 131.03, 134.24, 134.81, 136.62, 137.71, 140.82, 148.61, 169.72; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{30}H_{22}N_4OCl_2$: 524.1171; found (%): 524.1190.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(4-dimethylaminobenzylidene)acetohydrazide (4e): IR (KBr, ν_{max}, cm^{-1}): 3230 (N-H), 3074 (Ar C-H), 2915 (C-H), 1690 (C=O), 1592 (C=N), 1238 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 3.01 (6H, s, N-$(CH_3)_2$), 4.56 and 4.98 (2H, 2s, N-CH_2-CO), 6.62 (2H, d, *J* = 8.5 Hz, *m,m*'-Ar-H on benzylidene ring), 7.14-7.24 (3H, 2t, *J* = 7.5 Hz, *p*-Ar-H on 2,4,5-phenyl rings), 7.36-7.49 (10H, m, Ar-H on 2,4,5-phenyl and benzylidene rings), 7.52 (1H, s, HC=N), 7.59 (2H, d, *J* = 7.5 Hz, *o*,*o*'-Ar-H on 5-phenyl ring), 7.77 (2H, d, *J* = 6.5 Hz, *o*,*o*'-Ar-H on 2-phenyl ring), 8.96 (1H, s, CONH); HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{32}H_{29}N_5O$: 499.2372; found (%): 499.2550.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(4-ethoxybenzylidene)acetohydrazide (4f): IR (KBr, ν_{max}, cm^{-1}): 3204 (N-H), 3061 (Ar C-H), 2981 and 2932 (C-H), 1683 (C=O), 1601 (C=N), 1256 (C-N), 1039 (C-O-C); ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 1.42 (3H, t, *J* = 7.0 Hz, CH_3), 4.04 (2H, q, *J* = 7.0 Hz, OCH_2), 4.50 and 4.99 (2H, 2s, N-CH_2-CO), 6.83 (2H, d, *J* = 7.0 Hz, *m*,*m*'-Ar-H on benzylidene ring), 7.15-7.24 (3H, 2t, *J* = 7.0 Hz, *p*-Ar-H on 2,4,5-phenyl rings), 7.39-7.47 (10H, m, Ar-H on 2,4,5-phenyl rings), 7.58 (2H, d, *J* = 7.0 Hz, *o,o*'-Ar-H on benzylidene ring), 7.62 (1H, s, HC=N), 7.76 (2H, dd, *J* = 1.5 Hz, *o,o*'-Ar-H of 2-phenyl ring), 9.92 (1H, s, CONH); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ_C: 14.70, 46.55, 63.62, 114.66, 125.44, 126.40, 127.01, 128.07, 128.24, 128.71, 128.90, 129.05, 129.09, 129.14, 129.39, 130.51, 130.62, 130.78, 131.02, 134.35, 137.58, 145.41, 148.59, 160.96, 169.69; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{32}H_{28}N_4O_2$: 500.2212; found (%): 500.2251.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(3,4-dimethoxybenzylidene)acetohydrazide (4g): IR (KBr, ν_{max}, cm^{-1}): 3199 (N-H), 3062 (Ar C-H), 2935 (C-H), 1685 (C=O), 1573 (C=N), 1269 (C-N), 1024 (C-O-C); ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 3.75 (6H, 3s, OCH_3), 4.97 and 4.54 (2H, 2s, N-CH_2-CO), 6.93 and 6.99 (1H, 2d, *J* = 9.0 Hz, m'-Ar-H of benzylidene ring), 7.07 (1H, d, *J* = 9.0 Hz, *o*'-Ar-H of benzylidene ring), 7.14 (2H, t, *J* = 7.0 Hz, *p*- and *o*-Ar-H of 2-phenyl and benzylidene rings), 7.22 (2H, t, *J* = 7.5 Hz, *p*-Ar-H of 4,5-phenyl rings), 7.39-7.50 (10H, m, Ar-H), 7.69 (2H, d, *J* = 7.0 Hz, *o,o*'-Ar-H of 2-phenyl ring), 7.79-8.26 (1H, 3s, HC=N), 11.41 and 11.51 (1H, 2s, CONH); HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{32}H_{28}N_4O_3$: 516.2161; found (%): 516.2150.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(3-ethoxy-4-methoxybenzylidene)acetohydrazide (4h): IR (KBr, ν_{max}, cm^{-1}): 3223 (N-H), 3059 (Ar C-H), 2929 (C-H), 1689 (C=O), 1602 (C=N), 1266 (C-N), 1032 (C-O-C); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 1.29 (3H, t, *J* = 6.75 Hz, -CH_3), 3.72 and 3.77 (3H, 2s, OCH_3), 3.97-4.04 (2H, m, OCH_2), 4.54 and 4.97 (2H, 2s, N-CH_2-CO), 6.91 and 6.97 (1H, 2d, *J* = 7.0 and 10.0 Hz, *m*'-Ar-H of benzylidene ring), 7.05 (1H, d, *J* = 8.0 Hz, *o*'-Ar-H of benzylidene ring), 7.14 (2H, t, *J* = 7.0 Hz, *p*- and *o*-Ar-H of 2-phenyl and benzylidene rings), 7.21 (2H, t, *J* = 8.0 Hz, p-Ar-H of 4,5-phenyl rings), 7.39-7.49 (10H, m, Ar-H), 7.69 (2H, d, *J* = 8.0 Hz, *o,o*'-Ar-H of 2-phenyl ring), 7.79-8.26 (1H, 3s, HC=N), 11.41 and 11.51 (1H, 2s, CONH); ^{13}C NMR (125.75 MHz, DMSO-d_6) δ_C: 15.02, 46.77, 55.89, 64.22, 108.85, 109.28, 112.77, 121.79, 122.61, 126.58, 126.62, 126.74, 128.59, 128.95, 129.08, 129.14, 129.45, 129.54, 129.61, 130.78, 130.88, 130.93, 131.10, 131.20, 131.29, 134.88, 134.96, 136.81, 136.85, 145.21, 148.04, 148.27, 149.45, 149.52, 150.38, 150.58, 168.98; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{33}H_{30}N_4O_3$: 530.2318; found (%): 530.1082.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(3-phenylallylidene)acetohydrazide (4i): IR (KBr, ν_{max}, cm^{-1}): 3216 (N-H), 3057 (Ar C-H), 2928 (C-H), 1671 (C=O), 1626 (C=C), 1558 (C=N), 1247 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 4.82 and 4.51 (2H, 2s, N-CH_2-CO), 6.76 (1H, dd, *J* =

9.5 Hz, C=CH=C), 6.91-7.07 (1H, m, C=CH), 7.14 (1H, t, *J* = 7.25 Hz, *p*-Ar-H on benzylidene ring), 7.21 (2H, t, *J* = 7.5 Hz, *m,m*'-Ar-H on benzylidene ring), 7.28-7.53 (14H, m, Ar-H), 7.57 (1H, d, *J* = 7.0 Hz, HC=N), 7.64 and 7.69 (2H, dd, *J* = 7.5 Hz, *o*-Ar-H of 2-phenyl ring), 11.50 and 11.39 (1H, 2s, CONH); ^{13}C NMR (125.75 MHz, DMSO-d_6) δ_C: 46.74, 125.01, 126.54, 126.74, 127.53, 128.60, 128.94, 129.09, 129.17, 129.30, 129.34, 129.48, 129.60, 130.80, 130.88, 130.92, 131.05, 131.19, 131.28, 134.93, 136.05, 136.78, 140.08, 147.72, 148.01, 168.66; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{32}H_{26}N_4O$: 482.2107; found (%): 482.2200.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-[(naphthalen-2-yl)methylene]acetohydrazide (4j): IR (KBr, ν_{max}, cm^{-1}): 3213 (N-H), 3059 (Ar C-H), 1680 (C=O), 1551 (C=N), 1234 (C-N); ^{1}H NMR (500 MHz, $CDCl_3$, δ, ppm): 5.08 (2H, s, N-CH_2-CO), 7.17 (1H, t, *J* = 7.0 Hz, *p*-Ar-H of 2-phenyl ring), 7.24 (2H, t, *J* = 7.5 Hz, *p*-Ar-H of 4,5-phenyl rings), 7.36-7.54 (10H, m, Ar-H), 7.61 (2H, d, *J* = 7.0 Hz, Ar-H of naphthalene ring), 7.70 (1H, dd, *J* = 1.5 Hz, Ar-H of naphthalene ring), 7.76-7.84 (7H, m, Ar-H), 10.07 (1H, s, CONH); ^{13}C NMR (125.75 MHz, $CDCl_3$) δ_C: 46.58, 122.41, 126.47, 126.80, 127.00, 127.16, 127.40, 127.86, 128.12, 128.44, 128.63, 128.76, 128.97, 129.10, 129.12, 129.21, 129.32, 129.42, 129.53, 130.51, 130.60, 130.77, 131.05, 132.98, 134.37, 137.63, 145.65, 148.62, 169.88; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{34}H_{26}N_4O$: 506.2107; found (%): 506.2210.

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-[(thiophen-2-yl)methylene]acetohydrazide (4k): IR (KBr, ν_{max}, cm^{-1}): 3199 (N-H), 3056 (Ar C-H), 1686 (C=O), 1598 (C=N), 1236 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 4.85 and 4.53 (2H, 2s, N-CH_2-CO), 7.07 (1H, t, *J* = 4.5 Hz, C_4-H of thiophene ring), 7.12-7.23 (3H, 2t, *p*-Ar-H of 2,4,5-phenyl rings), 7.35 (1H, d, *J* = 4.0 Hz, C_3-H of thiophene ring), 7.39 (2H, d, *J* = 7.0 Hz, *o,o*'-Ar-H of 4-phenyl ring), 7.43-7.58 (8H, m, *m,m*'-Ar-H of 2,4,5-phenyl rings and *o,o*'-Ar-H of 2-phenyl ring), 7.56-7.71 (3H, m, *o*-Ar-H of 2-phenyl ring and C_5-H of thiophene ring), 8.04-8.26 (1H, 3s, HC=N), 11.46 and 11.57 (1H, 2s, CONH); ^{13}C NMR (125.75 MHz, DMSO-d_6) δ_C: 46.39, 126.53, 126.74, 126.79, 128.41, 128.59, 128.97, 129.09, 129.15, 129.27, 129.47, 129.57, 129.62, 129.72, 130.75, 130.86, 130.90, 131.03, 131.19, 131.29, 131.35, 131.89, 134.87, 134.93, 136.78, 136.81, 138.53, 138.79, 140.27, 143.21, 148.04, 148.07, 168.64; HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{28}H_{22}N_4OS$: 462.1514; found (%): 462.1600.

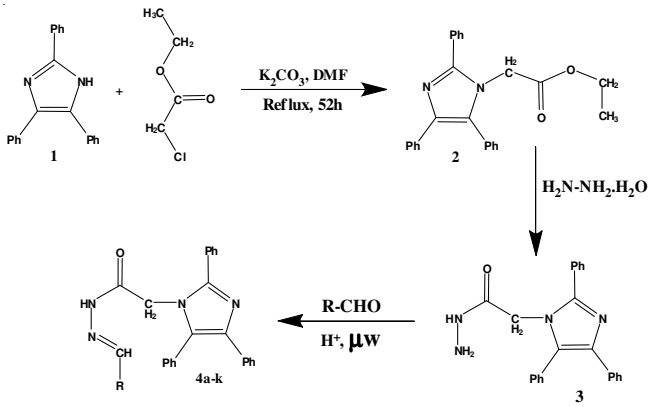
Procedure for the synthesis of (N',N'',N',N'')-N',N''-(1,4-phenylene*bis*(methan-1-yl-1-ylidene))*bis*(2-(2,4,5-triphenyl-1*H*-imidazol-1-yl)acetohydrazide) (5)

Method A: A mixture of compound **3** (0.01 mol) in chloroform/methanol (1:1v/v) mixture (30 mL), terephthalaldehyde (0.005 mol) and 1 mL of glacial acetic acid was refluxed on a water bath for 3 h. The mixture was allowed to cool and then the separated solid was filtered, washed with excess of methanol.

Method B: A mixture of compound **3** (0.01 mol) and terephthalaldehyde (0.005 mol) wetted with glacial acetic acid (2 drops) was ground by pestle in mortar at room temperature, followed by the addition of DMSO (2 mL). The resulting reaction mixture was subjected to microwave irradiation using microwave oven (MW domestic type oven 800W SANYO) at 20 % intensity for 3 min (6 pulses each of 30 s) and the product was set aside to cool. The progress of reaction was monitored by TLC. After completion of reaction, the reaction mixture was poured into ice-cold water. The separated product was filtered, washed with excess of cold water and dried at room temperature. m.p. 200-202 ºC; 56-66 % yield; IR (KBr, ν_{max}, cm^{-1}): 3396 (N-H), 3058 (Ar C-H), 1694 (C=O), 1204 (C-N); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm): 4.47 and 4.96 (4H, 2s, N-CH_2-CO), 7.14-7.23 (6H, m, *p*-Ar-H on 2,4,5-phenyl rings), 7.38-7.67 (28H, m, Ar-H), 7.84 and 7.87 (2H, 2s, HC=N), 11.58 and 11.65 (2H, 2s, CONH); ^{13}C NMR (125.75 MHz, DMSO-d_6) δ_C: 46.69, 126.54, 126.74, 127.70, 128.59, 128.95, 129.15, 129.56, 130.89, 131.19, 134.91, 136.80, 148.02, 169.16; LC-MS (m/z): 835.2 [M + 1], 836.2 [M + 2], 837.2 [M + 3]. HRMS (EI): m/z $[M]^+$ calcd. (%) for $C_{54}H_{42}N_8O_2$: 834.3431; found (%): 834.3480.

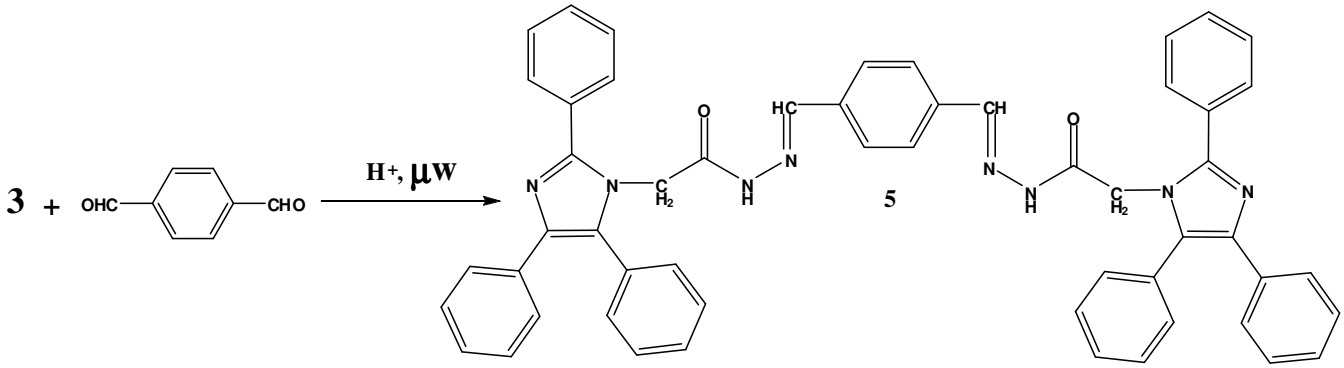
RESULTS AND DISCUSSION

2-(2,4,5-Triphenyl-1*H*-imidazol-1-yl)-N'-(substituted aryl)acetohydrazide (**4a-k**) were prepared through the intermediate 2-(2,4,5-triphenyl-1*H*-imidazol-1-yl)acetohydrazide (**3**) and different substituted aryl aldehydes in the ratio of 1:1 v/v chloroform to methanol under reflux temperature for 60-180 min/microwave irradiation for 2-3 min in DMSO using glacial acetic acid as a catalyst (**Scheme-I**) (Table-1).



Scheme-I: Synthetic protocol of compounds **4a-k**

The compound **5** was prepared by the reaction between intermediate **3** and terephthaladehyde under reflux temperature as well as microwave irradiation (**Scheme-II**). The intermediate 2-(2,4,5-triphenyl-1*H*-imidazol-1-yl)acetohydrazide (**3**) was prepared from ethyl 2-(2,4,5-triphenyl-1*H*-imidazol-1-yl)acetate (**2**), which was obtained by stirring 2,4,5-triphenyl-1*H*-imidazole (**1**) and ethylchloroacetate in DMF solvent at 75-80 ºC for 52 h using anhydrous K_2CO_3 as catalyst. The starting material 2,4,5-triphenyl-1*H*-imidazole (**1**) was obtained by reacting benzil, benzaldehyde and ammonium acetate in ethanol under reflux conditions using glacial acetic acid as a catalyst. All the synthesized compounds were confirmed by the spectral characterization through IR, ^{1}H NMR, ^{13}C NMR and mass spectral studies. These simple synthetic methods of new active heterocyclic derivatives with potential biological application would be of increasing interest to the synthetic chemists.



Scheme-II: Synthetic protocol of compound **5**

TABLE-2
ANTIOXIDANT ACTIVITY OF ALL THE SYNTHESIZED COMPOUNDS **4a-k** AND **5** USING DPPH RADICAL SCAVENGING METHOD

Antioxidant activity* (%) after 0.5 min incubation					
Compound	Absorbance	Antioxidant activity (%)	Compound	Absorbance	Antioxidant activity (%)
4a	0.203	65.99	**4g**	0.018	96.98
4b	0.148	75.21	**4h**	0.066	88.94
4c	0.168	71.86	**4i**	0.159	73.36
4d	0.059	90.11	**4j**	0.208	65.19
4e	0.042	92.96	**4k**	0.039	93.47
4f	0.051	91.45	**5**	0.131	78.06
Control**	0.597	–	–	–	–

*BHT was used as standard antioxidant. **DPPH in methanol.

Antioxidant activity: Compounds having antioxidant activity can minify the oxidative decay in foods, as well as prevent several related diseases, such as diabetes, cancer, Alzheimer's and Parkinson's diseases[26]. There are very few reports on antioxidant activity of synthetic compounds. All the synthesized compounds **4a-k** and **5** were subjected to antioxidant activity using DPPH free radical scavenging method. The antioxidant activity results are given inTable-2.

The DPPH (2,2-diphenyl-1-picrylhydrazyl radical, a stable free radical with characteristic absorption maxima at 517 nm) radical scavenging method is widely used to evaluate antioxidant activities in a relatively short period of time compared to other methods. The effect of antioxidants on DPPH radical scavenging is expected to be due to their hydrogen donating ability at a very rapid rate. The degree of discoloration indicates the scavenging activity of antioxidant (purple to yellow). Free radical scavenging activity of compounds **4a-k, 5** and BHT at 100 ppm concentration was tested by DPPH free radical scavenging method and the results are depicted in Table-3. Of the compounds **4a-k** and **5** the compound **4g** at a concentration of 100 ppm exhibited the highest radical scavenging activity of 96.98 % and the least of 65.19 % by the compound **4j**. The antioxidant activity for BHT, the commercially most used antioxidant, is measured as 100.00 % at similar concentration. Fig. 1 represents the observable effect of all the synthesized compounds **4a-k**, **5** and BHT on scavenging of free radicals. The data obtained bring out that the synthesized compounds are free radical inhibitors and primary antioxidants reacting with DPPH radical which may be assigned to its hydrogen donating ability. Radical scavenging activity was expressed as the inhibition percentage and was calculated using the following formula:

TABLE-3
PERCENTAGE OF VIABILITY OF CELLS TO THE COMPOUNDS **4a, 4c, 4e, 4g, 4k** AND **5**

Concentration of compound (μg/mL)	Percentage of viability of cells*					
	4a	**4c**	**4e**	**4g**	**4k**	**5**
5	42	62	48	40	68	40
15	28	41	32	29	39	28
25	21	36	24	21	34	20
50	19	21	19	18	20	18
100	18	19	16	15	18	14

*MTT assay method against liver cancer cell lines.

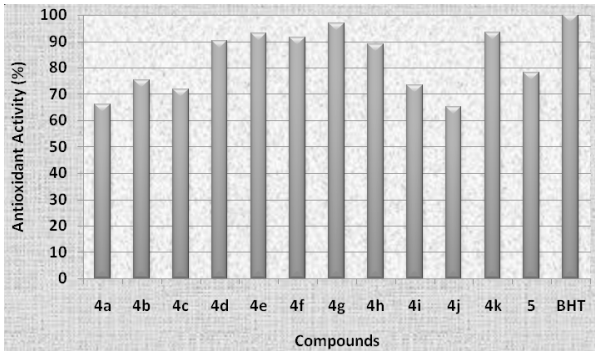


Fig. 1. Antioxidant activity (%) of compounds **4a-k** and **5** compared with BHT using DPPH radical scavenging method

$$\text{Radical scavenging activity (\%)} = \frac{[(\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Screening for antioxidant activity: The free radical scavenging activity was determined using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), as per procedure given by Willians *et al.*[27]. In brief, each compound (500 μL) containing 0.1 mg of soluble solids/mL were added to a methanolic

solution of DPPH (0.1 mmol, 3 mL). After a 0.5 h incubation period at the ambient temperature in dark, the absorbance was read at 517 nm. The control contained all the reaction reagents except the synthetic compound or positive control substance. The experiment was carried out in triplicate. The DPPH scavenging activity was expressed as the inhibition of free radical DPPH in per cent (I %) as described by Tepe *et al.*[28].

$$\text{Inhibition (\%)} = \frac{[\text{Control OD} - \text{Sample OD})}{\text{Control OD}} \times 100$$

Anticancer activity

MTT Assay: MTT Assay is a sensitive, quantitative and reliable assay for determining the number of viable cells in a given culture. It is a colorimetric assay based on conversion of a tetrazolium salt MTT, a pale yellow substrate to formazan, a purple dye. This cellular reduction involves the pyridine nucleotide cofactor NADH/NADPH and is only catalyzed by living cells. The formazan product has a low aqueous solubility and is present as purple crystals. It is dissolved with a solubilization buffer, which permits the convenient quantification of product formation. The intensity of the colour formed is measured in the range of 550-620 nm and this is directly proportional to the number of viable cells in the culture[29].

The compounds **4a, 4c, 4e, 4g, 4k** and **5** were screened for their anticancer activity against liver cancer cell line (**Hep G2**) using MTT assay method. Percentage of viability of cells was calculated for compounds **4a, 4c, 4e, 4g, 4k** and **5** at five different concentrations (5, 15, 25, 50 and 100 µg/mL). Viability of cells (%) at 100 µg/mL for compound **4a, 4c, 4e, 4g, 4k** and **5** is observed as 18, 19, 16, 15, 18 and 14, respectively. Among these compounds, compound **5** exhibited maximum activity of cell death at 5, 25, 50 and 100 µg/mL concentrations. This shows that compound **5** can be used as a good anticancer drug for killing the liver cancer cells. Fig. 2 shows the percentage viable cells at different concentrations of **4a**, **4c, 4e, 4g, 4k** and **5** when tested aganist liver cancer cells.

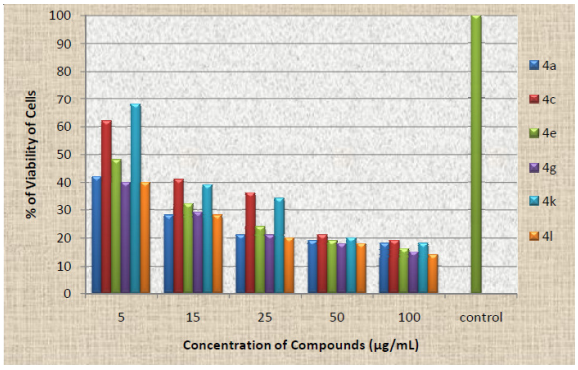


Fig. 2. Anticancer activity of compounds **4a, 4c, 4e, 4g, 4k** and **5** using MTT assay method

Screening for anticancer activity: Culture cells (80 µL) were plated in the 96 well culture plates. The number of cells can vary from 1000-80,000 per well. The volume of culture cell per well can vary from 50-150 µL and along with the culture cells there should be one control well containing culture medium without cells or cells treated with toxic reagent, such as 0.1 % saponin (Cell Quant -MTT TM Cell Viability Assay Kit, 2004). Test compounds were added to the wells and incubated overnight.

The MTT reagent and the assay buffer were reconstituted. First both were brought to room temperature. Then 1 mL of the assay buffer was added to the MTT Reagent tube, vortexed and the reconstituted solution was pipetted to the assay buffer bottle. It was repeated several times to completely mix the reagent. Then the bottle was labelled as reconstituted reagent. 15 µL of the reconstituted reagent was added to each well and incubated for 4 h at 37 ºC. The volume of the reagent was adjusted depending on the volume of the cell culture. 100 µL of the solubilisation solution was added per well. It was mixed for 1 h in an orbital shaker at room temperature. The volume of solubilisation solution was adjusted depending on the volume of the cell culture. The absorbance (O.D.) value was measured at 570 nm for each well on an absorbance plate reader. Cell viability (%) is calculated by the following formula.

Cell viability (%) = (Mean absorbance of sample/Mean absorbance of control) × 100

Conclusion

A series of novel Schiff base derivatives incorporating 2,4,5-triphenyl-1*H*-imidazole moiety have been synthesized under reflux conditions as well as microwave irradiation and screened for antioxidant activity using DPPH free radical scavenging method. All the compounds showed moderate to potent antioxidant activity. The compounds **4a, 4c, 4e, 4g, 4k** and **5** were subjected to check their anticancer activity against liver cancer cell lines (**Hep G2**) using MTT assay method. Among these compounds, compound **5** showed maximum activity of cell death at 5, 25, 50 and 100 µg/mL concentrations. This reported biological activity of these compounds should trigger more interest in the synthesis of such compounds from the easily available starting materials.

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REFERENCES

1. (a) D.J. Faulkner, *Nat. Prod. Rep.*, **17**, 1 (2000); (b) J.R. Lewis, *Nat. Prod. Rep.*, **19**, 223 (2002); (c) Z. Jin, Z. Li and R. Huang, *Nat. Prod. Rep.*, **19**, 454 (2002); (c) J.H. Ho, R.M. Hohareb, J.H. Ahn, T.B. Sim and H. Rapoport, *J. Org. Chem.*, **68**, 109 (2003).
2. (a) J.G. Lombardino and E.H. Wiseman, *J. Med. Chem.*, **17**, 1182 (1974); (b) J.G. Lombardino, Ger. Offen. 2155558 (1972); *Chem. Abstr.*, **77**, 1016074 (1972); (c) A.P. Phillips, H.L. White and S. Rosen, Eur. Pat. Appl. EP58890 (1982); *Chem. Abstr.*, **98**, 53894z (1983).
3. A.F. Pozherskii, A.T. Soldatenkov and A.R. Katritzky, Heterocycles in Life and Society, Wiley, New York, pp. 179-180 (1997).
4. R. Liebl, R. Handte, H. Mildenberger, K. Bauer and H. Bieringer, Ger. Offen. Pat., 3604042 (1987); *Chem. Abstr.*, **108**, 6018g (1987).
5. E.O. Renth, K. Schromm, R. Anderskewitz, F. Birke, A. Fuegner and H. Heuer, Patent WO 9421616 (1994); *Chem. Abstr.*, **122**, 81416c (1995).
6. D.C. Fenske, E.A. Kuo and W.R. Tully, US Patent 4810828 (1989); *Chem. Abstr.*, **109**, 170425d (1988).
7. P. Chiesi, V. Servadio and R. Razzetti, EP Patent 301422 (1989); *Chem. Abstr.*, **111**, 225320q (1989).
8. T. Nishi, T. Uno, Y. Koga and G.N. Chu, EP Patent 240015 (1987); *Chem. Abstr.*, **108**, 167318v (1988).

9. T. Maier, R. Schmierer, K. Bauer, H. Bieringer, H. Buerstell and B. Sachse, US Patent 820335 (1989); *Chem. Abstr*., **111**, 19494 (1989).
10. P. Renukadevi, J.S. Biradar, S.P. Hiremath and S.Y. Manjunath, *Indian J. Heterocycl. Chem*., **6**, 277 (1997).
11. L.C. Patricia, M.G. Christine and B.M. Stephen, *J. Agric. Food Chem*., **36**, 1076 (1988).
12. H. Miyachi, H. Kiyota and M. Segawa, *Bioorg. Med. Chem. Lett*., **18**, 2163 (1998).
13. T.R. Sharpe, S.C. Cherkovsky, W.E. Hewes, D.H. Smith, W.A. Gregory, S.B. Haber, M.R. Leadbetter and J.G. Whitney, *J. Med. Chem*., **28**, 1188 (1985).
14. I. Sircar, B.L. Duell, A. Bristol, R.E. Weishaar and D.B. Evans, *J. Med. Chem*., **30**, 1023 (1987).
15. S.C. Jewery, G. Danswan, C.R. Gardner, S.S. Matharu, R. Murdoch, W.R. Tully and R. Westwood, *J. Med. Chem*., **31**, 1220 (1988).
16. T. Kimura, Y. Takase, K. Hayashi, H. Tanaka, I. Ohtsuka, T. Saeki, M. Kogushi, T. Yamada and T. Fujimori,, *J. Med. Chem*., **36**, 1630 (1993).
17. C. Chan, E.J. Bailey, C.D. Hartley, D.F. Hayman, J.L. Hutson, G.G.A. Inglis, P.S. Jones, S.E. Keeling and B.E. Kirk, *J. Med. Chem*., **36**, 3646 (1993).
18. P. Cozzi, G. Carganico, D. Fusar, M. Grossoni, M. Menichincheri, V. Pinciroli, R. Tonani, F. Vaghi and P. Salvati, *J. Med. Chem*., **36**, 2964 (1993).
19. J. Mohan and A. Kumar, *Indian J. Heterocycl. Chem*., **12**, 41 (2002).
20. Y.F. Shealy, J.A. Montogomery and W.R.J. Loster, *Biochem. Pharmacol*., **11**, 674 (1962).
21. S. Meenakshi, R. Reenakalsi, K.S. Dixit, C. Nath and J.P. Barthwal, *Indian J. Chem*., **29B**, 85 (1990).
22. M. Harfenist, E.F. Soroko and G.M. Mekenzie, *J. Med. Chem*., **21**, 405 (1978).
23. V.S.V. Satyanarayana, P. Sreevani, A. Sivakumar and V. Vijayakumar, *ARKIVOC*, 221 (2008).
24. L. Jin, J. Chen, B. Song, Z. Chen, S. Yang, Q. Li, D. Hu and R. Xu, *Bioorg. Med. Chem. Lett*., **16**, 5036 (2006).
25. (a) R.J. Giguere, T.L. Bray, S.M. Duncan and G. Majetich, *Tetrahedron Lett*., **27**, 4945 (1986); (b) R. Gedye, F. Smith, K. Westaway, H. Ali, L. Baldisera, L. Laberge and J. Rousell, *Tetrahedron Lett*., **27**, 279 (1986).
26. L. Dalby-Brown, H. Basett, A.K. Landbo, A. Meyer and P. Molgaard, *J. Agric. Food Chem*., **53**, 9413 (2005).
27. W. Brand-Willians, M.E. Cuvelier and C. Berset, *Food Sci. Tech*., **28**, 25 (1995).
28. B. Tepe, M. Sokmen, H.A. Akpulat and A. Sokmen, *Food Chem*., **95**, 200 (2006).
29. Wallert and Provost Lab, Proliferation Assay MTT Protocol, Minnesota State University Moorhead (2004).