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Design, Synthesis and Anti-cancer Evaluation of some Novel Leucettamine B Analouges

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ABSTRACT

Objective: Novel anticancer agents were designed to be synthesized considering Leucettamine B as a lead compound. Leucettamine B, which was isolated from a marine sponge, is a tyrosine-regulated inhibitor of kinase 1A (Dyrk1A), and has gained a lot of attention as a pharmacological target in malignant brain tumors. **Method:** 3-Phenyl-2-thioxoimidazolidin-4-one **1** has been used for the synthesis of various fused phenylimidazo[1,2-f]pyrimidine **4**, phenylimidazo[2,1-b]quinazoline **6**, phenylimidazo[1,2-b][1,2,4]triazine **10-14, 20** and **21** as well as imidazo[1,2-b][1,2,4]triazepine analogues **15** and **17** through different chemical reactions. **Results**: Considering that eleven of the synthesized compounds were selected for evaluation of their anticancer activity by the National Cancer Institute (NCI), U.S.A. The anticancer evaluation was carried out against National Cancer Institute (NCI) cell lines using single high dose. **Conclusion:** Compounds 8-(4-Methoxybenzylidene)-2,4-diamino-5-phenyl-5H-imidazo[1,2-b][1,2,4,6] tetrazepin-7(8H)-one **16, 20** and **24** were found to have selective potent anticancer activity against Non-Small Cell Lung cancer EKVX, Melanoma UACC-62 and Breast cancer HS 578T cell lines.

Keywords: *Anticancer, Imidazotriazine, imidazotriazepine, Leucettamine.*

INTRODUCTION

2-Thioxoimidazolidin-4-one and its derivatives consist of an important class of heterocyclic compounds due to its wide variety of pharmacological activities as; Hypolipidemic [1], anti-cancer [2,3], anti-viral [4,5], anti-tuberculosis [6], anti-microbial (anti-fungal and anti-bacterial) [7], anti-ulcer and anti-inflammatory [8].

Leucettamine B, which was isolated from a marine sponge, is a tyrosine-regulated inhibitor of kinase 1A (Dyrk1A) and has gained a lot of attention as a pharmacological target in malignant brain tumors.[9]

It is worth mentioning that. Marino, M. et al [9] indicated the essential presence of 2-aminoimidazolidine carbonyl oxygen for the activity of Leucettamine B derivatives. Therefore, it was designed to synthesize novel anticancer agents comprising the essential features

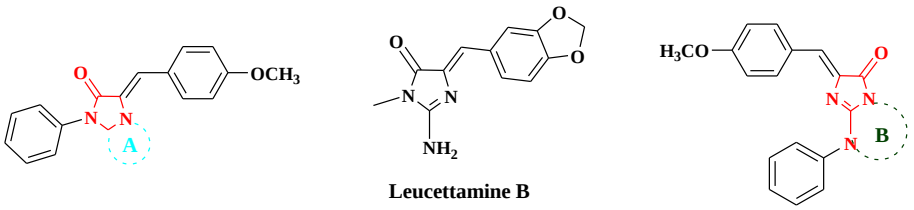


Figure 1. Feature resemblance between the target compounds and Leucettamine B.

for anticancer activity considering Leucettamine B as a lead compound **Figure 1**. 3-Phenyl-2-thioxoimidazolidin-4-one **1**, was utilized as building units to synthesize various innovative agents prone to combat cancer.

MATERIAL AND METHODS

Part 1-Chemistry:

All melting points were determined in open glass capillaries on Electro thermal LA 9000 SERIS and are uncorrected. IR spectra were recorded, for potassium bromide discs, on Nikolet IR 200 FT IR spectrophotometer at pharmaceutical analytical unit, Faculty of pharmacy, Al-Azhar University, and the values are represented in cm-1. **^{1}H NMR** spectra were determined on Varian Gemini EM-400 MHz, NMR spectrometer at laboratories of nuclear magnatic resonance, Chemical Warefare Department, Ministery of Defense. DMSO-d_6 was used as solvent; chemical shifts were measured in δ ppm, relative to TMS as an internal standard. Mass spectra were carried out using a Schimadzu GC/ MS-QP-5050A mass spectrometer at 70 ev at Regional Center for Mycology and biotechnology, Al-Azhar University. Elemental analyses were performed on Elementar Vario EI III CHN analyzer at Micro-analytical, Regional Center for Mycology and biotechnology, Al-Azhar University. Progress of the reactions were monitored by thin-layer chromatography (TLC) on silica gel sheets (60 GF 254, Merk), the spots were visualized by exposure to UV-lamp at λ 254 and 365 nm for few seconds.

2-(4-(4-Methoxybenzylidene)-5-oxo-1-phenylimidazolidin-2-ylidene)-2-cyanoacetamide (3)

A solution of 5-(4-methoxybenzylidene)-3-phenyl-2-thioxoimidazolidin-4-one [10,11] **2** (0.62 g, 2 mmol.) in absolute ethanol (10) and 2-cyanoacetamide (0.17 g, 2 mmol.) mixture in pyridine (10 mL) was refluxed for 6 hours till evolution of H_2S ceased. After cooling to room temperature, the reaction mixture was then poured in ice cooled water, the precipitated solid formed was filtered off, dried and crystallized from ethanol to afford compound **3**.

Yellow crystals, **yield;** 0.52 g (84 %), **m.p.**; >360 °C. **IR (KBr, cm^{-1}):** 3335, 3221 (NH_2, NH); 3044, 2936 (CH-aromatic); 2843 (CH-aliphatic); 2197 (C≡N); 1723 (C=O); 1644 (C=N); 1599, 1518 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 3.30 (s, 2H, NH_2, D_2O exchangeable); 3.80 (s, 3H, OCH_3, under DMSO); 6.65 (s, 1H, CH-benzylidene); 6.98-7.20 (m, 1H, N-C_6H_5-C_4-H); 7.36 (d, 2H, *J*=8.6 Hz, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.39-7.52 (m, 2H, N-C_6H_5-$C_{3,5}$-H); 7.66 (d, 2H, *J*= 8.8 Hz, N-C_6H_5-$C_{2,6}$-H); 7.81 (d, 2H, *J*=8.6 Hz, 4-OCH_3-C_6H_4-$C_{2,6}$-H); 12.49 (s, 1H, imidazole-NH, D_2O exchangeable). **MS:** m/z (%): 361 (M+1, 2.01); 360 ($M^{+\cdot}$, 3.18); 359 (M-1, 2.52); 66 (100). **Anal. form:** ($C_{20}H_{16}N_4O_3$). **Calcd. (%):** C, 66.66; H, 4.48; N, 15.55. **Found (%):** C, 66.89; H, 4.60; N, 15.81.

3-(4-Methoxybenzylidene)-1,2,3,5,6,7-hexahydro-2,5,7-trioxo-1-phenylimidazo[1,2-f] pyrimidine-8-carbonitrile (4)

A mixture of 2-[4-(4-methoxybenzylidene)-5-oxo-1-phenylimidazolidin-2-ylidene]-2-cyanoacetamide **3** (0.72 g, 2 mmol.) and ethyl chloroformate (0.22 g, 0.19 mL, 2 mmol.) in ethanol containing sodium ethoxide (30 mL) was heated under reflux for 4 hours. The reaction mixture was left to cool, poured onto crushed ice, the obtained precipitate was filtered, washed with water, dried, and crystallized from ethanol to afford compound **4**.

Pale yellow crystals, **yield;** 0.30 g (45 %), **m.p.;** 263-265 °C. **IR (KBr, cm^{-1}):** 3434 (OH tautomer); 3189 (NH); 3000 (CH-aromatic); 2988 (CH-aliphatic); 2250 (C≡N); 1739 (C=O); 1571 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 3.73 (s, 3H, OCH_3); 5.54 (s, 1H, pyrimidine-NH, D_2O-exchangble); 6.39 (s, 1H, CH-benzylidene); 6.40-7.46 (m, 1H, N-C_6H_4-C_4-H); 6.55 (d, 2H, *J*=8 Hz, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.09 (d, 2H, *J*=8 Hz, 4-OCH_3-C_6H_5-$C_{2,6}$-H); 7.37-7.41 (m, 2H, N-C_6H_5-$C_{3,5}$-H); 7.79 (d, 2H, *J*= 8.4 Hz, N-C_6H_5-$C_{2,6}$-H). **^{13}C NMR (DMSO-*d6*, δ ppm):** 55.86 (OCH_3); 76.51 (imidazopyrimidine-C_8); 113.97 (4-OCH_3-C_6H_4-$C_{3,5}$); 114.85 (CN); 114.91 (benzylidene-C); 125.37 (N-C_6H_5-$C_{2,6}$); 125.75 (N-C_6H_5-C_4); 127.31 (4-OCH_3-C_6H_4-C_1); 129.17 (4-OCH_3-C_6H_4-$C_{2,6}$); 129.26 (N-C_6H_5-$C_{3,5}$); 129.35 (imidazopyrimidine-C_3); 131.81 (N-C_6H_5-C_1); 132.82 (imidazopyrimidine-C_5); 133.88 (4-OCH_3-C_6H_4-C_4);

160.90 (imidazopyrimidine-C_2); 164.40 (imidazopyrimidine-C_7); 178.46 (imidazopyrimidine-C_{8a}). **MS:** m/z (%): 388 (M+2, 7.31); 387 (M+H, 30.12); 386 ($M^{+\cdot}$, 2.17); 385 (M-1, 1.22); 310 (100). **Anal. form:** ($C_{21}H_{14}N_4O_4$). **Calcd. (%):** C, 65.28; H, 3.65; N, 14.50. **Found (%):** C, 65.49; H, 3.72; N, 14.73.

3-(4-Methoxybenzylidene)-10-phenylimidazo[1,2-f]benzo[e][1,2,4]thiadiazine-2-(*1H, 10H, 10a H*)one (5)

5-(4-Methoxybenzylidene)-3-phenyl-2-thioxoimidazolidin-4-one **2** (0.62 g, 2 mmol.) and 2-aminothiophenol (0.25 g, 0.21 mL, 2 mmol.) in ethanol containing sodium ethoxide (30 mL) has been heated under reflux for 20 hours before revolution of H_2S ceased. The reaction mixture was condensed, allowed to reach room temperature, poured on crushed ice, the solid obtained was filtered and hot ethanol washed the obtained precipitate.

Yellow crystals, **yield**; 0.32 g (52 %), **m.p.** >360 °C. **IR (KBr, cm^{-1}):** 3182 (NH); 3066, 3032 (CH-aromatic); 2924 (CH-aliphatic); 1724 (C=O); 1635 (C=N); 1508, 1600 (C=C). **MS:** m/z (%): 400 (M-1, 13.95); 41 (100). **Anal. form:** ($C_{23}H_{19}N_3O_2S$). **Calcd. (%):** C, 68.81; H, 4.77; N, 10.47; S, 7.99. **Found (%):** C, 69.48; H, 4.38; N, 10.81; S, 8.14.

3-(4-Methoxybenzylidene)-8-methoxy-1-phenylimidazo[2,1-b]quinazoline-2,5(1H,3H)-dione; (6)

A mixture of 5-(4-methoxybenzylidene)-3-phenyl-2-thioxoimidazolidin-4-one **2** (0.62 g, 2 mmol.) and 4-methoxyanthranilic acid (0.33 g, 2 mmol.) in ethanol containing sodium ethoxide (30 mL), it was heated for 20 hours under reflux before the evolution of H2S stopped. The reaction mixture was condensed, allowed to reach room temperature, poured onto ice, filtered, washed with water, dried and ethanol-crystallized the obtained precipitate.

Brown powder, **yield;** 0.48 g (77 %), **m.p.;** >360 °C **IR (KBr, cm^{-1}):** 3066 (CH-aromatic); 2920 (CH-aliphatic); 1743, 1680 (C=O); 1604 (C=N); 1570, 1512 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 3.74 (s, 3H, OCH_3); 3.77 (s, 3H, OCH_3); 6.10 (s, 1H, CH-benzylidene); 6.31-6.35 (m, 1H, N-C_6H_4-C_4-H); 6.47 (d, 2H, *J*= 8 Hz, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 6.85 (d, 2H, *J*= 8 Hz, 4-OCH_3-C_6H_5-$C_{2,6}$-H); 7.20-7.22 (m, 2H, N-C_6H_5-$C_{3,5}$-H); 7.34-7.42 (m, 2H, imidazoquinazoline-$C_{6,7}$-H); 7.70 (d, 2H, *J*= 9.2 Hz, N-C_6H_5-$C_{2,6}$-H); 8.10 (d, 2H, *J*= 8.8 Hz, imidazoquinazoline-C_9-H). **^{13} C NMR (DMSO-*d6*, δ ppm):** 55.79 (OCH_3); 55.91 (OCH_3); 114.60 (imidazoquinazoline-C_9); 114.79 (imidazoquinazoline-C_{5a}); 114.86 (imidazoquinazoline-C_6); 114.93 (4-OCH_3-C_6H_4-$C_{3,5}$); 126.62 (N-C_6H_5-$C_{2,6}$); 127.01 (benzylidene-C); 127.25 (N-C_6H_5-C_4); 129.29 (4-OCH_3-C_6H_4-C_1); 129.35 (4-OCH_3-C_6H_4-$C_{2,6}$); 129.76 (imidazoquinazoline-C_5); 130.11 (N-C_6H_5-$C_{3,5}$); 131.61 (N-C_6H_5-C_1); 132.52 (imidazoquinazoline-C_{9a}); 156.28 (4-OCH_3-C_6H_4-C_4); 160.89 (imidazoquinazoline-C_{11}); 161.73 (imidazoquinazoline-C_2); 162.02 (imidazoquinazoline-$C_{5,8}$). **Anal. form:** ($C_{25}H_{19}N_3O_4$). **Calcd. (%):** C, 70.58; H, 4.50; N, 9.88. **Found (%):** C, 70.79; H, 4.72; N, 10.07.

2-Ethyl-3-methyl-6-(4-methoxybenzylidine)-4,7-dioxo-8-phenyl(4H,6H,7H, 8H)imidazo[1,2-a]thieno[2,3-d] pyrimidine-2-carboxylate (8)

A mixture of 5-(4-methoxybenzylidene)-3-phenyl-2-thioxoimidazolidin-4-one **2** (0.62 g, 2 mmol.) and diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate **7** [12] in ethanol containing sodium ethoxide (30 mL) was heated for 20 hours under reflux. The reaction mixture was condensed, cooled, and poured onto ice, and the collected precipitate was filtered, washed with ethanol and dried.

Grey powder; yield 0.45 g (73 %) m.p. 278-280 °C. **IR (KBr, cm^{-1}):** 3062, 2951 (CH-aromatic); 2920, 2850 (CH-aliphatic); 1735, 1700 (C=O); 1627 (C=N); 1600, 1512 (C=C). **MS:** m/z (%): 489 (M+2, 19.20); 488 (M+H, 6.15); 487 ($M^{+\cdot}$, 27.83); 390 (100). **Anal. Form:** ($C_{26}H_{21}N_3O_5S$). **Calcd. (%):** C, 64.05; H, 4.34; N, 8.62; O, 16.41; S, 6.58. **Found (%):** C, 64.21; H, 4.49; N, 8.78; S, 6.49.

6-(4-methoxybenzylidene)-2-methyl-4-phenylimidazo[1,2-b][1,2,4]triazine-3,7(4H,6H)-dione (10)

A mixture of 5-(4-methoxybenzylidene)-2-(phenylamino)-*1H*-imidazol-4(*5H*)-one **9** [13] (0.62 g, 2 mmol.) and pyruvic acid (0.18 g, 0.14 mL, 2 mmol.) in absolute ethanol (20 mL) was heated under reflux for 6 hours. The reaction mixture was condensed, cooled, filtered, dried and crystallized from acetone with the obtained precipitate.

Yellow crystals, **yield;** 0.40 g (56 %), **m.p.;** 280 °C. **IR (KBr, cm^{-1}):** 3000, 2975 (CH-aromatic); 2941 (CH-aliphatic); 1723 (C=O); 1635 (C=N); 1603, 1566 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 3.87 (s, 6H, CH3 & OCH_3); 7.15-7.19 (m, 5H, N-C_6H_5-$C_{2,3,4,5,6}$-H); 7.93-7.98 (m, 4H, 4-OCH_3-C_6H_4-C-H); 8.37 (s, 2H, CH-benzylidene). **^{13} C NMR (DMSO-*d6*, δ ppm):** 33.40 (CH_3); 55.79 (OCH_3); 114.42 (4-OCH_3-C_6H_4-$C_{3,5}$); 114.79 (N-C_6H_5-$C_{2,6}$); 127.25 (N-C_6H_5-C_4); 129.76 (4-OCH_3-C_6H_4-$C_{1,2,6}$); 133.19 (benzylidene-C); 133.85 (N-C_6H_5-$C_{3,5}$); 134.73 (N-C_6H_5-C_1); 156.29 (imidazotriazine-C_7); 160.32 (imidazotriazine-C_2); 161.73 (4-OCH_3-C_6H_4-C_4); 165.87 (imidazotriazine-C_3); 174.90 (imidazotriazine-C_{4a}); 178.75 (imidazotriazine-C_6). **Anal. form:** ($C_{20}H_{16}N_4O_3$). **Calcd. (%):** C, 66.66; H, 4.48; N, 15.55. **Found (%):** C, 66.94; H, 4.62; N, 15.80.

6-(4-Methoxybenzylidene)-4-phenylimidazo[1,2-b][1,2,4]triazine-2,3,7(1H,4H,6H)-trione (11)

Method A:

5-(4-Methoxybenzylidene)-2-(phenylamino)-*1H*-imidazol-4(*5H*)-one **9** (0.62 g, 2 mmol.) and oxalyl chloride (0.25 g, 0.17 mL, 2 mmol.) mixture in absolute ethanol (20 mL) for 6 hours, it was heated under reflux. The reaction mixture was condensed, cooled, filtered, dried and recrystallized from ethanol to obtain well-formed needle crystals.

Method B:

Compound **9** (0.62 g, 2 mmol.) was stirred in excess diethyl oxalate (0.29 g, 0.27 ml, 2 mmol.) at room temperature for 2 hours. The formed crystals were collected, recrystallized from ethanol and dried.

Coluorless needle crystals, **yield;** 0.4 g (55 %), **m.p.**; 156-160 °C. **IR (KBr, cm^{-1}):** 3425 (OH tautomer); 3053 (CH-aromatic); 2938 (CH-aliphatic); 1712 (C=O); 1608 (C=N); 1512, 1491 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 4.27 (s, 3H, OCH_3); 7.25 (d, 4H, *J*= 7.2 Hz, 4-OCH_3-C_6H_4-C-H); 7.38-7.50 (m, 6H, CH-benzylidene & N-C_6H_5-C-H); 10.41 (s, 1H, OH tautomer, D_2O exchangeable). **^{13} C NMR (DMSO-*d6*, δ ppm):** 55.79 (OCH_3); 114.79 (4-OCH_3-C_6H_4-$C_{3,5}$); 122.65 (N-C_6H_5-$C_{2,6}$); 122.91 (N-C_6H_5-C_4); 123.43 (4-OCH_3-C_6H_4-$C_{2,6}$); 127.25 (4-OCH_3-C_6H_4-C_1 & benzylidene-C); 129.76 (N-C_6H_5-$C_{3,5}$); 130.63 (N-C_6H_5-C_1); 153.42 (imidazotriazine-C_7); 153.93 (imidazotriazine-C_2); 154.12 (imidazotriazine-C_3); 156.29 (4-OCH_3-C_6H_4-C_4); 161.73 (imidazotriazine-C_{4a}); 166.44 (imidazotriazine-C_6). **MS:** m/z (%): 363 (M+1, 34.15); 362 ($M^{+\cdot}$, 58.04); 361 (M-1, 27.63); 351 (100). **Anal. form:** ($C_{19}H_{14}N_4O_4$). **Calcd. (%):** C, 62.98; H, 3.89; N, 15.46. **Found (%):** C, 63.14; H, 4.01; N, 15.68.

7-(4-Methoxybenzylidene)-3,4-diphenylimidazo[1,2-b][1,2,4]triazin-6(1H,4H,7H)-one (12)

A mixture of 5-(4-methoxybenzylidene)-2-(phenylamino)-*1H*-imidazol-4(*5H*)-one **9** (0.62 g, 2 mmol.) and phenacyl bromide (0.4 g, 2 mmol.) in sodium ethoxide (30 mL) was heated for 6 hours, cooled, poured onto crushed ice, the obtained product was then crystallized from ethanol and dried.

Yellow crystals, **yield;** 0.40 g (49 %), **m.p.;** 205 °C. **IR (KBr, cm^{-1}):** 3381 (NH); 3097, 3034 (CH-aromatic); 2922, 2852 (CH-aliphatic); 1713 (C=O); 1649 (C=N); 1584, 1555 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 3.70 (s, 3H, OCH_3); 4.25 (s, 1H, imidazotriazine-C_3-H); 7.62-7.73 (m, 6H, CH-benzylidene & C_6H_5-C-H); 7.91-7.96 (m, 7H, 4-OCH_3-C_6H_5-C-H & N-C_6H_5-$C_{3,4,5}$-H); 8.52-8.54 (m, 2H, N-C_6H_5-$C_{2,6}$-H) 9.99 (s, 1H, triazine-NH, D_2O exchangeable);. **^{13} C NMR (DMSO-*d6*, δ ppm):** 55.77 (OCH_3); 112.90 (imidazotriazine-C_2); 114.42 (4-OCH_3-C_6H_4-$C_{3,5}$); 114.71 (N-C_6H_5-$C_{2,6}$); 114.84 (N-C_6H_5-C_4); 120.96 (C_6H_5-$C_{2,6}$); 125.64 (4-OCH_3-C_6H_4-$C_{2,6}$); 126.59 (4-OCH_3-C_6H_4-C_1); 127.30 (C_6H_5-C_4); 128.54 (C_6H_5-$C_{3,5}$); 128.97 (benzylidene-C); 129.16 (N-C_6H_5-$C_{3,5}$); 131.81 (C_6H_5-C_1); 132.60 (N-C_6H_5-C_1); 160.32 (imidazotriazine-C_7); 161.09 (4-OCH_3-C_6H_4-C_4); 165.87 (imidazotriazine-C_{3a}); 174.90 (imidazotriazine-C_6). **MS:** m/z (%): 411 (M+3, 14.28); 410 (M+2, 45.76); 409 (M+H, 14.70); 408 ($M^{+\cdot}$, 3.89); 308 (100). **Anal. form:** ($C_{25}H_{20}N_4O_2$). **Calcd. (%):** C, 73.51; H, 4.94; N, 13.72. **Found (%):** 73.82; H, 4.78; N, 13.89.

2-Methyl 2-(-6-(4-methoxybenzylidene)-3,4,6,7-tetrahydro-3,7-dioxo-4-phenylimidazo[1,2-b][1,2,4]triazin-2(1H)-ylidene)acetate (13)

A mixture of compound **9** (0.62 g, 2 mmol.) and dimethyl acetylene dicarboxylate (0.28 g, 0.24 ml, 2 mmol.) in absolute ethanol (10 mL) was heated under reflux for 20 hours. The precipitated solid was filtered, dried and crystallized from ethanol.

Dark grey crystals, **yield;** 0.50 g (60 %), **m.p.;** 178-180 °C **IR (KBr, cm^{-1}):** 3131 (NH); 3038 (CH-aromatic); 2805 (CH-aliphatic); 1685 (C=O); 1596 (C=N); 1407 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 3.31 (m, 6H, two OCH_3, under DMSO); 4.22 (s, 1H, CH=$COOCH_3$); 7.10 (s, 1H, CH-benzylidene); 7.21 (d, 2H, J= 6.8 Hz, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.25-7.50 (m, 7H, N-C_6H_5-C-H & 4-OCH_3-C_6H_4-$C_{2,6}$-H); 10.40 (s, 1H, NH-triazine, D_2O exchangeable). **Anal. form:** ($C_{22}H_{18}N_4O_5$). **Calcd. (%):** C, 63.15; H, 4.34; N, 13.39. **Found (%):** C, 63.49; H, 4.47; N, 13.60.

2-(-6-(4-methoxybenzylidene)-3,4,6,7-tetrahydro-7-oxo-4-phenylimidazo[1,2-b][1,2,4]triazin-3-yl)acetic acid (14)

A mixture of 5-(4-methoxybenzylidene)-2-(phenylamino)-*1H*-imidazol-4(*5H*)-one **9** (0.62 g, 2 mmol.) and maleic anhydride (1.96 g, 2 mmol.) was heated under reflux in absolute ethanol (10 mL) containing piperidine (0.17 g, 0.20 ml, 2 mmol.) for 4 hours. The reaction mixture was then poured into ice-cold water and hydrochloric acid was neutralized, filtered, crystallized from and dried the precipitated substance.

White crystals, **yield;** 0.58 g (77 %), **m.p.;** 188-190 °C. **IR (KBr, cm^{-1}):** 3444 (OH); 3000 (CH-aromatic); 2974, 2885 (CH-aliphatic); 1724 (C=O); 1627 (C=N); 1604, 1566 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm**): 3.73 (s, 3H, OCH_3); 3.75-3.82 (m, 1H, imidazotriazine C_3-H & CH_2COOH); 6.85-6.89 (m, 1H, imidazotriazine-C_2-H); 6.90-7.00 (m, 2H, N-C_6H_4-C_4-H); 7.04 (d, 2H, *J*= 8.8 Hz, 4-OCH_3-C_6H_5-$C_{3,5}$-H); 7.36-7.41 (m, 2H, N-C_6H_5-$C_{3,5}$-H);7.56-7.66 (m, 2H, N-C_6H_5-$C_{2,6}$-H); 7.76 (d, 2H, *J*= 8.8 Hz, 4-OCH_3-C_6H_4-$C_{2,6}$-H); 8.61 (s, 1H, CH-benzylidene); 11.60 (s, 1H, OH, D_2O exchangeable). **^{13} C NMR (DMSO-*d6*, δ ppm):** 33.39 (CH_2COOH); 55.79 (imidazotriazine-C_3); 55.84

(OCH_3); 114.79 (N-C_6H_5-$C_{2,6}$); 114.85 (4-OCH_3-C_6H_4-$C_{3,5}$); 127.02 (N-C_6H_5-C_4); 127.25 (4-OCH_3-C_6H_4-$C_{2,6}$); 128.87 (4-OCH_3-C_6H_4-C_1); 129.76 (benzylidene-C); 130.43 (N-C_6H_5-$C_{3,5}$); 132.26 (N-C_6H_5-C_1); 139.91 (imidazotriazine-C_7); 156.30 (imidazotriazine-C_2); 160.94 (4-OCH_3-C_6H_4-C_4); 161.73 (imidazotriazine-C_{4a}); 162.13 (imidazotriazine-C_6); 180.05 (C=O). **Anal. form:** ($C_{21}H_{18}N_4O_4$). **Calcd. (%):** C, 64.61; H, 4.65; N, 14.35. **Found (%):** C, 64.89; H, 4.73; N, 14.59.

2-(4-methoxybenzylidene)-9-phenyl-2H-imidazo[1,2-b][1,2,4]triazepine-3,6,8(5H,7H,9H)-trione (15)

5-(4-Methoxybenzylidene)-2-(phenylamino)-*1H*-imidazol-4(*5H*)-one **9** (0.62 g, 2 mmol.) and diethyl malonate (3.20 g, 3.05 mL, 2 mmol.) mixture was heated in absolute ethanol (10 mL) containing piperidine (0.17 g, 0.20 ml, 2 mmol.) under reflux for 30 hours. The reaction mixture was then poured onto ice cold water, neutralized with drops of hydrochloric acid and the precipitated product was filtered, crystallized from ethanol and dried.

Black crystals, **yield**; 0.30 g (40 %), **m.p.**; 158 °C. **IR (KBr, cm^{-1}):** 3429 (OH tautmer); 3321 (NH); 3093, 3066, 3035 (CH-aromatic); 2989, 2900 (CH-aliphatic); 1716 (C=O); 1612 (C=N); 1589, 1566 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 3.15 (s, 2H, imidazotriazipine-C_7-H); 4.01 (s, 3H, OCH_3); 6.92-6.99 (m,3H, N-C_6H_5-$C_{3,4,5}$-H); 7.85-7.90 (m, 4H, 4-OCH_3-C_6H_4-C-H); 8.28-8.30 (m, 3H, N-C_6H_5-$C_{2,6}$-H & CH-benzylidene); 11.04 (s, 1H, triazepine-NH, D_2O exchangeable). **MS:** m/z (%): 376 ($M^{+\cdot}$, 23.77); 92 (100). **Anal. form:** ($C_{20}H_{16}N_4O_4$). **Calcd. (%):** C, 63.82; H, 4.28; N, 14.89. **Found (%):** C, 63.61; H, 4.35; N, 15.12.

8-(4-Methoxybenzylidene)-2,4-diamino-5-phenyl-5H-imidazo[1,2-b][1,2,4,6] tetrazepin-7(8H)-one (16)

A mixture of 5-(4-methoxybenzylidene)-2-(phenylamino)-*1H*-imidazol-4(*5H*)-one **9** (0.62 g, 2 mmol.) and cyanoguanidine (0.17 g, 2 mmol.) was heated in water (20 mL) containing concentrated hydrochloric acid for 6 hours under reflux. The reaction mixture was then condensed, allowed to cool and the precipitated solid was filtered, washed with water, dried and crystallized from ethanol.

Yellow crystals, **yield;** 0.42 g (56 %), **m.p.;** 105 °C. **IR (KBr, cm^{-1}):** 3321, 3178 (NH_2); 3032, 3012 (CH-aromatic); 2920 (CH-aliphatic); 1678 (C=O); 1604 (C=N); 1554, 1512 (C=C).**^{1}H NMR (DMSO-*d6*, δ ppm):** 3.11 (s, 3H, OCH_3); 6.02 (s, 4H, two NH_2-tetrazepine, D_2O exchangeable); 7.25-7.32 (m, 3H, N-C_6H_5-$C_{3,4,5}$-H); 7.38-7.45 (m, 4H, 4-OCH_3-C_6H_4-C-H); 7.50-7.57 (m, 2H, N-C_6H_5-$C_{2,6}$-H); 8.12 (s, 1H, CH-benzylidene). **MS:** m/z (%): 378 (M+3, 41.75); 377 (M+2, 44.64); 376 (M+H, 55.76); 375 ($M^{+\cdot}$, 60.73); 361 (100). **Anal. form:** ($C_{19}H_{17}N_7O_2$). **Calcd. (%):** C, 60.79; H, 4.56; N, 26.12. **Found (%):** C, 61.03; H, 4.69; N, 26.42.

2-(4-methoxybenzylidene)-6-(5-bromo-2-hydroxyphenyl)-5,6-dihydro-8-methyl-9-phenyl-2H-imidazo[1,2-b][1,2,4]triazepin-3(9H)-one (17)

A solution of acetone (0.15 g, 0.22 mL, 3 mmol.) in dioxane (10 mL) containing a catalytic quantity of concentrated hydrochloric acid (2 mL) at room temperature, a mixture of 5-(4-methoxybenzylidene)-2-(phenylamino)-1H-imidazol-4(5H)-one **9** (0.62 g, 2 mmol.) and 5-bromosalicaldehyde (0.20 g, 2 mmol.) in methanol (10 mL) was added. The reaction mixture was heated for 25 hours under reflux, condensed, cooled and filtered with the precipitated solid, washed with methanol, dried.

Yellow powder, **yield;** 0.49 g (46 %), **m.p.;** >360 °C. **IR (KBr, cm^{-1}):** 3454 (OH); 3158 (NH); 3050, 3010 (CH-aromatic); 2920, 2785 (CH-aliphatic); 1725 (C=O); 1634 (C=N); 1598, 1502 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 1.89 (s, 3H, CH_3); 3.75-3.85 (m, 3H, OCH_3); 4.64-4.66 (m, 1H, imidazotriazepine-C_6-H); 6.48 (s, 1H, imidazotriazepine-C_7-H); 6.98-6.99 (m, 2H, N-C_6H_5-$C_{3,5}$-H); 7.03 (d, 1H, *J*= 8.8 Hz, C_6H_3(OH)Br-C_3-H); 7.30-7.37 (m, 1H, N-C_6H_5-C_4-H); 7.60 (d, 2H, *J*= 8.8 Hz, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.46-7.52 (m, 2H, N-C_6H_5-$C_{2,6}$-H); 7.94 (s,1H, C_6H_3(OH)Br-C_6-H); 7.77-7.86 (m, 1H, C_6H_3(OH)Br-C_4-H); 7.68 (d, 2H, , *J*= 8.8 Hz, 4-OCH_3-C_6H_4-$C_{2,6}$-H); 8.61 (s, 1H, CH-benzylidene); 10.19 (s, 1H, triazepine-NH, D_2O exchangeable); 11.43 (s,1H, OH, D_2O exchangeable). **^{13}C NMR (DMSO-*d6*, δ ppm):** 55.73 (CH_3); 55.77 (OCH_3); 55.90 (imidazotriazepine-C_6); 108.46 (imidazotriazepine-C_7); 114.48 (4-OCH_3-C_6H_4-$C_{3,5}$); 114.74 (C_6H_3(OH)Br-C_5); 114.85 (N-C_6H_5-$C_{2,6}$); 119.25 (C_6H_3(OH)Br-C_3); 119.39 (N-C_6H_4-C_4); 125.74 (C_6H_3(OH)Br-C_1); 126.43 (4-OCH_3-C_6H_4-$C_{2,6}$); 127.02 (4-OCH_3-C_6H_4-C_1); 128.14 (benzylidene-C); 129.25 (N-C_6H_5-$C_{3,5}$); 131.65 (C_6H_3(OH)Br-C_4); 133.08 (C_6H_3(OH)Br-C_6); 135.39 (imidazotriazepine-C_8); 135.95 (N-C_6H_5-C_1); 152.89 (imidazotriazepine-C_3); 155.51 (C_6H_3(OH)Br-C_2); 158.11 (4-OCH_3-C_6H_4-C_4); 163.045 (imidazotriazepine-C_{9a}); 163.94 (imidazotriazepine-C_2). **MS:** m/z (%): 532 (M+2, 2.97); 530 ($M^{+\cdot}$, 5.16); 529 (M-1, 1.51); 450 (100). **Anal. form:** ($C_{27}H_{23}BrN_4O_3$). **Calcd.** C, 61.03; H, 4.36; Br, 15.04; N, 10.54. **Found (%):** C, 61.27; H, 4.52; N, 10.71.

5-(4-Methoxybenzylidene)-2-(2-chlorophenyl)-2,3-dihydro-3-phenyl-1H-imidazo[1,2-b][1,2,4]triazol-6(5H)-one (18)
And 2-(4-Methoxybenzylidene)-11-phenyl-6H-imidazo[1,2-b]benzo[e][1,2,4] triazepine-3-one (19)

A mixture of 5-(4-methoxybenzyllidene)-2-(phenylamino)-1H-imidazole-4(5H)-one **9** (0.62 g, 2 mmol.) and 2-chlorobenzaldehyde (0.28 g, 0.22 ml, 2

mmol.) in dry benzene (10 mL) containing triethyl amine (0.20 g, 0.26 mL, 2 mmol.) has been heated for 10 hours under reflux. To reach room temperature, the reaction mixture was left and the acquired precipitate was filtered to yield compound **18**, while the filtrate was condensed to supply compound **19.**

5-(4-Methoxybenzylidene)-2-(2-chlorophenyl)-2,3-dihydro-3-phenyl-1H-imidazo[1,2-b][1,2,4]triazol-6(5H)-one (18)

Yellow crystals, **yield**; 0.36 g (42 %), **m.p.**; 163-165 °C. **IR (KBr, cm^{-1}):** 3350 (NH); 3097, 3034 (CH-aromatic); 1716 (C=O); 1584 (C=C); 1489 (C=N). **^{1}H NMR (DMSO-*d*$_6$, δ ppm):** 3.31 (s, 3H, OCH_3, under DMSO); 3.65 (s, 1H, imidazotriazole C_2-H); 6.55 (s, 1H, triazole-NH, D_2O exchangeable); 6.99 (d, 2H, *J*= 7.2 Hz, 2-Cl-C_6H_4-C_3-H); 7.04 (d, 2H, *J*= 8.8 Hz, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.33 (d, 1H, *J*= 7.2 Hz, 2-Cl-C_6H_4-C_6-H); 7.35-7.53 (m, 3H, N-C_6H_5-$C_{3,4,5}$-H); 7.89 (d, 2H, *J*= 8.8 Hz, 4-OCH_3-C_6H_4-$C_{2,6}$-H); 7.67-7.71 (m, 2H, 2-Cl-C_6H_4-$C_{4,5}$-H); 7.87 (d, 1H, *J*= 8.8 Hz, N-C_6H_5-$C_{2,6}$-H); 8.61 (s, 1H, CH-benzylidene). **^{13}C NMR (DMSO-*d*$_6$, δ ppm):** 55.80 (OCH_3); 57.98 (imidazotriazole-C_2); 114.80 (N-C_6H_4-$C_{2,6}$); 117.69 (4-OCH_3-C_6H_4-$C_{3,5}$); 120.62 (N-C_6H_5-C_4); 126.63 (2-Cl-C_6H_4-C_5); 127.01 (4-OCH_3-C_6H_4-$C_{2,6}$); 127.25 (4-OCH_3-C_6H_4-C_1); 129.26 (2-Cl-C_6H_4-C_4); 129.76 (2-Cl-C_6H_4-C_6); 130.11 (2-Cl-C_6H_4-C_3); 130.36 (benzylidene-C); 130.43 (N-C_6H_5-$C_{3,5}$); 131.61 (2-Cl-C_6H_4-C_2); 132.25 (2-Cl-C_6H_4-C_2); 145.72 (N-C_6H_5-C_1); 161.73 (imidazotriazole-C_6); 164.21 (4-OCH_3-C_6H_4-C_4); 166.81 (imidazotriazole-C_{3a}); 166.82 (imidazotriazole-C_5). **MS:** m/z (%): 431 (M+1, 31.61); 430 ($M^{+\cdot}$, 42.33); 429 (M-1, 31.26); 421 (100). **Anal. form:** ($C_{24}H_{19}ClN_4O_2$). **Calcd. (%):** C, 66.90; H, 4.44; N, 13.00. **Found (%):** C, 67.13; H, 4.61; N, 13.29.

2-(4-Methoxybenzylidene)-11-phenyl-6H-imidazo[1,2-b]benzo[e][1,2,4] triazepine-3-one (19)

Brown crystals, **yield;** 0.20 g (25 %), **m.p.;** >360 °C. **IR (KBr, cm^{-1}):** 3070, 3022 (CH-aromatic); 2975 (CH-aliphatic); 1724 (C=O); 1636 (C=N); 1603, 1565, 1509 (C=C). **^{1}H NMR (DMSO-*d*$_6$, δ ppm):** 3.31 (s, 3H, OCH_3); 7.18 (s, 1H, CH-benzylidene); 7.20-7.22 (m, 2H, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.24 (s, 1H, triazepine-C_3-H); 7.40-7.45 (m, 3H, N-C_6H_5-$C_{3,4,5}$-H); 7.56-7.61 (m, 4H, C_6H_4-C-H); 7.64-7.67 (m, 2H, 4-OCH_3-C_6H_4-$C_{2,6}$-H); 8.08-8.12 (m, 2H, N-C_6H_5-$C_{2,6}$-H). **Anal.form:** ($C_{24}H_{18}N_4O_2$). **Calcd. (%):** C, 73.08; H, 4.60; N, 14.20. **Found (%):** C, 73.36; H, 4.72; N, 14.39.

6-(4-Methoxybenzylidene)-3-methyl-4-phenylimidazo[1,2-b][1,2,4]triazin-7(1H,4H,6H)-one (20) and 1-(2-Oxopropylamino)-5-(4-methoxybenzylidene)-2-(phenylamino)-1H-imidazol-4(5H)-one (21)

A mixture of 5-(4-methoxybenzylidene)-2-(phenylamino)-1H-imidazol-4(5H)-one **9** (0.62 g, 2 mmol.) and chloroacetone (0.18 g, 0.16 mL, 2 mmol.) in sodium ethoxide (30ml) was heated for 6 hours under reflux. The reaction mixture was left to reach room temperature, poured on crushed ice, and filtered and crystallized the formed precipitate from methanol to yield compound 20, while the filtrate was condensed.

6-(4-Methoxybenzylidene)-3-methyl-4-phenylimidazo[1,2-b][1,2,4]triazin-7(*1H,4H,6H*)-one (20)

Dark grey powder, **yield**; 0.24 g (35 %), **m.p.**; >360 °C. **IR (KBr, cm^{-1}):** 3228 (NH); 3086, 3012 (CH-aromatic); 2920 (CH-aliphatic); 1678 (C=O); 1608 (C=C); 1556 (C=N); 1238, 1029 (C-O-C). **MS:** m/z (%): 349 (M+3, 2.86); 348 (M+2, 1.73), 347 (M+H, 14.96), 345 (M-1, 5.86); 152 (100). **Anal.form:** ($C_{20}H_{18}N_4O_2$). **Calcd. (%):** C, 69.35; H, 5.24; N, 16.17. **Found (%):** C, 69.57; H, 5.38; N, 16.41.

1-(2-Oxopropylamino)-5-(4-methoxybenzylidene)-2-(phenylamino)-*1H*-imidazol-4(*5H*)-one (21)

Brown powder, **yield;** 0.55 g (76 %), **m.p.;** 180-182 °C. **IR (KBr, cm^{-1}):** 3323 (NH); 3050, 2965 (CH-aromatic); 2922, 2852 (CH-aliphatic); 1679 (C=O); 1604 (C=N); 1556 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 1.09 (s, 3H, CH_3); 4.14 (s, 2H, CH_2); 4.57 (s, 3H, OCH_3); 7.28 (s, 1H, NH , D_2O exchangeable); 7.40-7.44 (m, 1H, N-C_6H_5-$C_{2,4,6}$-H); 7.49-7.52 (m, 2H, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.69-7.76 (m, 2H, 4-OCH_3-C_6H_4-$C_{2,6}$-H); 8.36 (s, 1H, CH-benzylidene); 11.78 (s, 1H, NH-C_6H_5, D_2O exchangeable). **MS:** m/z (%): 364 ($M^{+\cdot}$, 17.47); 45 (100). **Anal.form:** ($C_{20}H_{20}N_4O_3$). **Calcd. (%):** C, 65.92; H, 5.53; N, 15.38. **Found (%):** C, 65.74; H, 5.79; N, 15.70.

5-(4-Methoxybenzylidene)-2-hydroxy-3-phenyl-3H-imidazo[1,2-b][1,2,4]triazol-6(5H)-one (22)

Compound **9**(0.62 g, 2 mmol.) and ethyl chloroformate (0.22 g, 0.19 ml, 2 mmol.) in 30 ml sodium ethoxide was refluxed nfor 5 hours. The reaction mixture was cooled, poured on crushed ice, filtered, washed with water, dried and crystallized from ethanol to the product obtained.

Pale yellow crystals, **yield;** 0.30 g (45 %), **m.p.;** 125 °C. **IR (KBr, cm^{-1}):** 3455 (OH); 2956 (CH-aromatic); 2852 (CH-aliphatic); 1686 (C=O); 1654 (C=N); 1573 (C=C). **^{1}H NMR (DMSO-*d*$_6$, δ ppm):** 3.89 (s, 3H, OCH_3); 6.33 (s, 1H, CH-benzylidene); 6.95 (d, 2H, *J*= 8.4 Hz, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.65-7.68 (m, 3H, N-C_6H_4-$C_{3,4,5}$-H); 7.77 (d, 2H, *J*= 8.4 Hz, 4-OCH_3-C_6H_4-$C_{2,6}$-H); 8.12-8.14 (m, 2H, N-C_6H_5-$C_{2,6}$-H); 11.62 (s, 1H, OH, D_2O exchangble). **^{13}C NMR (DMSO-*d*$_6$, δ ppm):** 55.78 (OCH_3); 114.78 (4-OCH_3-C_6H_4-$C_{3,5}$); 118.09 (N-C_6H_4-$C_{2,6}$); 118.22 (N-C_6H_4-C_4); 127.25 (N-C_6H_5-$C_{3,5}$); 129.76 (4-OCH_3-C_6H_4-$C_{1,2,6}$); 130.12 (benzylidene-C); 134.20 (N-C_6H_5-C_1); 156.29 (imidazotriazole-C_6);

161.73 (4-OCH_3-C_6H_4-C_4); 162.75 (imidazotriazole-C_2); 164.42 (imidazotriazole-C_{3a}); 174.55 (imidazotriazole-C_5). **Anal. form:** ($C_{18}H_{14}N_4O_3$). **Calcd. (%):** C, 64.66; H, 4.22; N, 16.76. **Found (%):** C, 64.51; H, 4.38; N, 17.02.

Ethyl 2-(4-(4-methoxybenzylidene)-4,5-dihydro-5-oxo-2-(phenylamino)imidazol-1-ylamino)-2-cyano-5-phenylpent-4-enoate (24)

5-(4-Methoxybenzylidene)-2-(phenylamino)-1H-imidazole-4(5H)-one **9** (0.62 g, 2 mmol.) and ethyl 2-cyano-5-phenylpenta-2,4-dienoate **23**[14] (0.92 g, 2 mmol.) were heated under reflux in absolute ethanol (30 mL) containing piperidine (0.18 g, 0.20 mL, 1 mmol.) for 5 hours. The mixture of the reaction was cooled, poured onto crushed ice, neutralised with hydrochloric acid and filtered, washed with water, dried and ethanol crystallised the precipitate.

Yellow crystals, **yield;** 0.51 g (52 %), **m.p.;** >360 °C. **IR (KBr, cm^{-1}):** 3335 (NH); 3070 (CH-aromatic); 2955, 2919 (CH-aliphatic); 2206 (C≡N); 1736 (C=O); 1643 (C=N); 1601, 1572 (C=C). **^{1}H NMR (DMSO-*d6*, δ ppm):** 1.45-1.63 (m, 3H, $OCH_2\underline{CH_3}$); 2.99 (s, 2H, $\underline{CH_2}$-CH=CH-C_6H_5); 3.66-3.86 (m, 5H, $O\underline{CH_2}CH_3$ & OCH_3); 3.37 (s, 1H, N-NH, D_2O exchangeable); 6.56 (s, 1H, NH-ph, D_2O exchangeable); 6.97 (s, 1H, CH_2-$\underline{CH}$=CH-C_6H_5); 7.00 (s, 1H, CH_2-CH=$\underline{CH}$-C_6H_5); 7.03 (d, 2H, *J*= 8.8 Hz, 4-OCH_3-C_6H_4-$C_{3,5}$-H); 7.13-7.25 (m, 2H, two C_6H_5-C_4-H); 7.30-7.52 (m, 4H, two C_6H_5-$C_{3,5}$-H); 7.79 (d, 2H, *J*= 8.8 Hz, 4-OCH_3-C_6H_4-$C_{2,6}$-H); 7.83-7.89 (m, 2H, C_6H_5-$C_{2,6}$-H); 8.61 (s, 1H, CH-benzylidene). **Anal. form:** ($C_{32}H_{31}N_5O_4$). **Calcd. (%):** C, 69.93; H, 5.69; N, 12.74. **Found (%):** C, 69.70; H, 5.87; N, 12.98.

Part 2- Biology

Development therapeutic program (DTP), division of cancer treatment and diagnosis (DCTD), national cancer institute (NCI), Bethesda, Maryland, USA has adopted an in-vitro model consisting of 60 human tumor cell lines for primary anticancer screening [15]. Screening utilizes 60 different human tumor cell lines, representing leukemia, melanoma and cancers of the lung, colon, brain, ovary, breast, prostate, and kidney. It is a unique screen in that the complexity of a 60 cell line dose response produced by a given compound results in a biological response pattern which can be utilized in pattern recognition algorithms (COMPARE program).
Screening is a two-stage process, beginning with evaluation of all compounds against the 60 cell lines at single dose level of 10 μ mol.

The output from the single 60 cell panel screen is reported as a mean graph and is available for analysis by the COMPARE program.

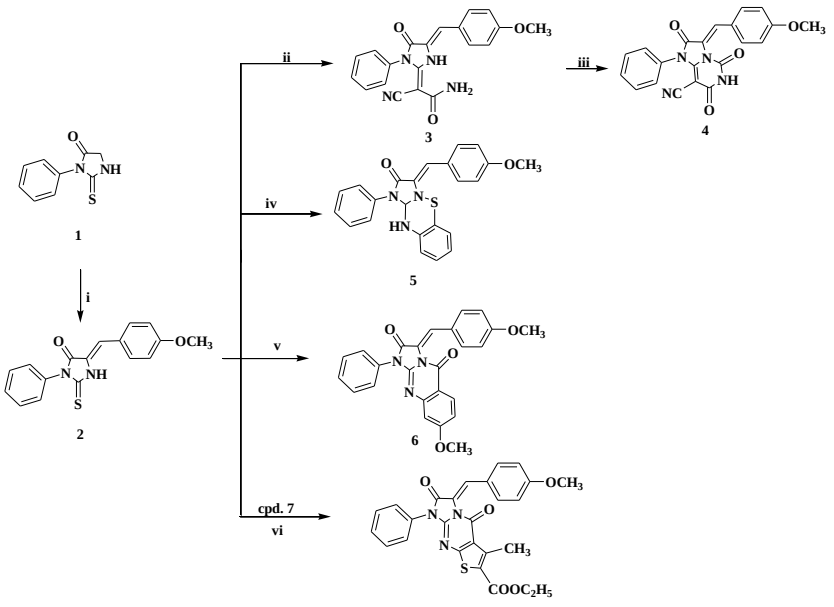
Compounds which inhibit growth by more than 50% in a threshold number of cell lines was determined by comparison with historical NCI 60 cell and in–vivo data (COMPARE program), were selected for 5-dose assay.

Mean graph is a mean of presenting the in-vitro test results to emphasize differential effects of test compounds on various human tumor cell lines. It plots the growth relative to no drug control and relative to time zero number of cells. Thus, it detects both growth inhibition (values between 0-100) and lethality (values less than 0) i.e a value of 40 means 60% growth inhibition while value of -40 means 40% lethality. The mean is the average of growth across the tested cell lines, while delta is the maximum difference from the mean.
Cell suspensions which were diluted according to the particular cell type and the expected target cell density were added into micro titer plates. Inoculates were allowed a pre-incubation period of 24 hrs at 37 0C for stabilization. Test compounds were then added at a single concentration (10 μmole) and culture were incubated for 48 hrs at 370 C in 5% CO2 atmosphere and 100% relative humidity. [15] Sulforhodamine B (SRB) protein assay was used to estimate cell viability or growth. SRB is a bright pink anionic dye that, in dilute acetic acid, would bind to the basic amino acids. This reagent gives the best combination of stain intensity, single-to-noise ratio and linearity with cell number. A plate reader was used to read the optical densities and microcomputer preceded the optical densities into special concentration parameters. Results of each compound were reported as a mean graph of the percent growth of treated cells relative to untreated control cell.

RESULTS AND DISCUSSION

Part 1-Chemistry

5-(4-Methoxybenzylidene)-3-phenyl-2-thioxoimidazolidin-4-one **2** was prepared adopting the reported methods [10,11] **"Scheme I"**. Compound **2** was reacted with cyanoacetamide in absolute ethanol/pyridine mixture to yield the cyanoacetamide derivative **3** which was further reacted with ethyl chloroformate in sodium ethoxide to yield the cyclic imidazopyrimidine derivative **4.** **^{1}H NMR** spectrum of compound **3** displayed two deuterium oxide exchangeable singlet signals at δ 3.30 and 12.49 ppm attributed to amino and NH imidazole protons; respectively, while **^{1}H NMR** spectrum of compound **4** revealed singlet signal at δ 5.54 ppm attributed to NH pyrimidine proton which disappeared by deuteriation. Reaction of compound **2** with 2-aminothiophenol in sodium ethoxide which was suggested to yield first the open chain intermediate, in which the imidazole-NH function carried out intramolecular electrophilic addition of the thiol group on the imine double bond to yield the cyclic imidazo[1,2-f]benzo[e][1,2,4]thiadiazine **5**



Reagents and conditions: (i) 4- Methoxybenzaldehyde/ CH_3COONa/ gl. acetic acid/ reflux; **(ii)** Cyanoacetamide/ absolute ethanol/ pyridine/ reflux; **(iii)** Ethyl chloroformate/ $NaOC_2H_5$./ reflux; **(iv)** 2-Aminothiophenol / $NaOC_2H_5$./ reflux; **(vi)** 4-methoxyanthranilic acid / $NaOC_2H_5$./ reflux; **(vii)** Diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate **7**/ $NaOC_2H_5$/ reflux.

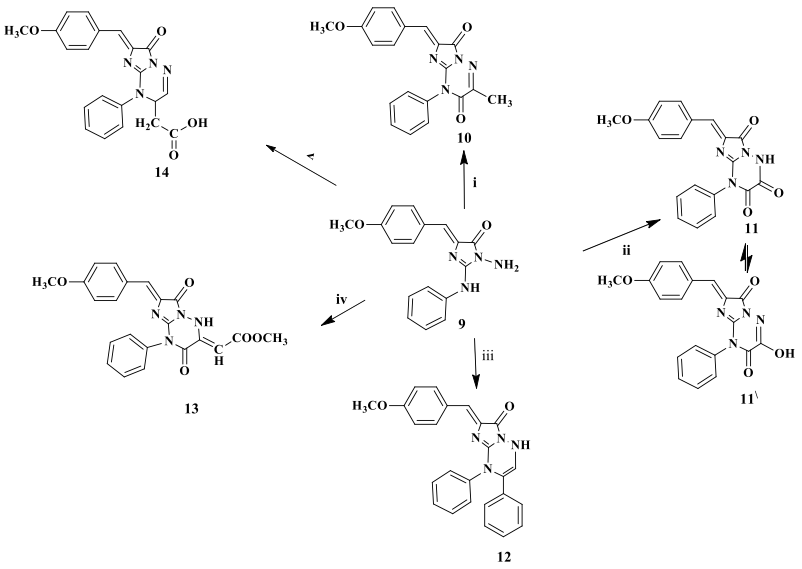
Scheme 1

of the thiol group on the imine double bond to yield the cyclic imidazo[1,2-f]benzo[e][1,2,4]thiadiazine **5**. Compound **2** was also reacted with 4-methoxyanthranilic acid in sodium ethoxide to yield the imidazoquinazoline analogue **6**. Two singlets at δ 3.74, 3.77 ppm corresponding to two methoxy protons as well as multiplet at δ 7.34-7.42 ppm attributed to quinazoline-$C_{6,7}$ protons were detected in **^{1}H NMR** spectrum of compound 6. Furthermore, compound **8** was prepared through the reaction of compound **2** with diethyl 5-amino-3-methylthiophene-2,4-dicarboxylate **7** [12].

The target compound **9** was prepared using the reported method [13] and was utilized as starting material for preparation of **scheme II**. Compound 9 was reacted with pyruvic acid in absolute ethanol to give the imidazotriazine derivative **10** "**Sceheme II". ^{1}H NMR** spectrum of compound **10** displayed one singlet signal at 3.87 ppm due to methyl and methoxy protons. Also, the target imidazotriazindione derivative **11** was obtained by reacting compound **9** with either oxalyl chloride in absolute ethanol via heating under reflux or by stirring with excess diethyl oxalate at room temperature. The obtained compound **11** via the two methods was confirmed to be the same compound via TLC, m.p. and mixed m.p. **^{1}H NMR** spectrum of compound **11** showed a deuterium oxide exchangeable singlet signal at δ 10.41 ppm due to hydroxyl proton.

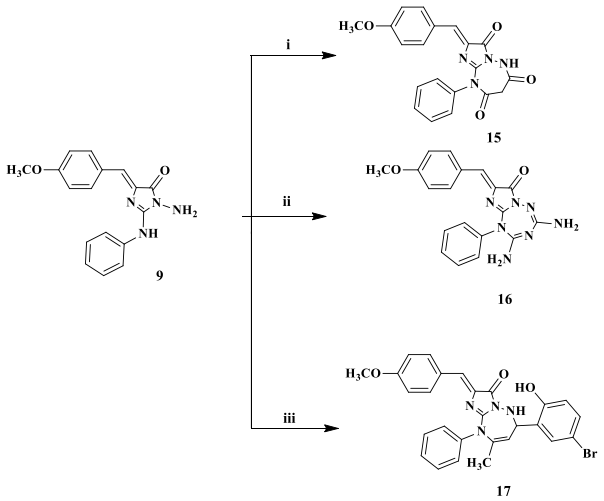
The cyclic imidazotriazine derivative **12** was obtained via the reaction of compound **9** with phenacyl bromide in sodium ethoxide to yield the cyclic. A deuterium oxide exchangeable singlet signal at δ 9.99 ppm corresponding to NH triazine proton, beside to a singlet signal at δ 4.25 ppm due to imidazotriazine-C_3 proton in **^{1}H NMR** spectrum confirmed the structure of compound **12.** Compound **9** and dimethyl acetylene dicarboxylate was heated under reflux in absolute ethanol to yield the imidazotriazine derivative **13**. The **^{1}H NMR** spectrum of compound **13** displayed a singlet signal at δ 4.22 ppm due CH-$COOCH_3$ proton and a deuterium oxide exchangeable singlet signal at δ 10.40 ppm corresponding to NH-triazine proton. Compound **9** was also heated under reflux with equimolar amount of maleic anhydride in absolute ethanol containing a catalytic amount of piperidine to yield the target imidazotriazine derivatives **14**. **^{1}H NMR** spectrum of compound **14** displayed a deuterium oxide exchangeable singlet signal at δ 11.60 ppm corresponding to carboxylic proton.

Our scope was extended to study the effect of fusion of seven membered rings to the imidazole nucleus. So, compound **9** was heated under reflux with an equimolar amount of diethyl malonate in absolute ethanol containing a catalytic amount of piperidine to yield the cyclic imidazotriazepine derivative **15 "Scheme III". ^{1}H NMR** spectrum of compound **15** displayed a singlet signal at δ 3.15 ppm due to imidazotriazipine C_7 protons and a deuterium oxide exchangeable singlet signal at δ 11.04 ppm corresponding to NH-triazipine proton. Moreover,



Reagents and conditions: **(i)** Pyruvic acid/ absolute ethanol/ reflux; **(ii)** Oxalyl chloride/ absolute ethanol/ reflux; or diethyloxalate/ stirring/ RT; **(iii)** Phenacyl bromide/ $NaOC_2H_5$./ reflux; **(iv)** DMAD/ absolute ethanol/ reflux; **(v)** Maliec anhydride/ absolute ethanol/ piperidine/ reflux.

Scheme II



Reagents and conditions: **(i)** Diethyl malonate/ absolute ethanol/ piperidine./ reflux; **(ii)** Cyanoguanidine/ H_2O/ c. HCl/ reflux; **(iii)** 5-bromosalicyldehyde/ acetone/ methanol/ dioxane/ c.HCl/ reflux.

Scheme III

compound **9** was heated under reflux with an equimolar amount of cyanoguandine in water containing a catalytic amount of concentrated hydrochloric acid to yield the cyclic imidazotetrazepine derivative **16**. The reaction mechanism was assumed to proceed through the nucleophilic addition of the amino function on the cyano group of the cyanoguanidine followed by subsequent cyclization through elimination of one molecule of ammonia. **^{1}H NMR** spectrum of compound **16** displayed a deuterium oxide exchangeable singlet signal at δ 6.02 ppm due to two tetrazepine amino protons.

Compound **9** was also treated with equimolar amounts of 5-bromosalicyaldehyde and acetone in methanol/ dioxane mixture containing a catalytic amount of concentrated hydrochloric acid to yield the cyclic imidazotriazepine derivative **17**. **^{1}H NMR** spectrum of compound **17** displayed two multiplet signals at δ 4.23-4.26 ppm and δ 4.64-4.66 ppm due to imidazotriazepine-C_6 and C_7 protons; respectively.

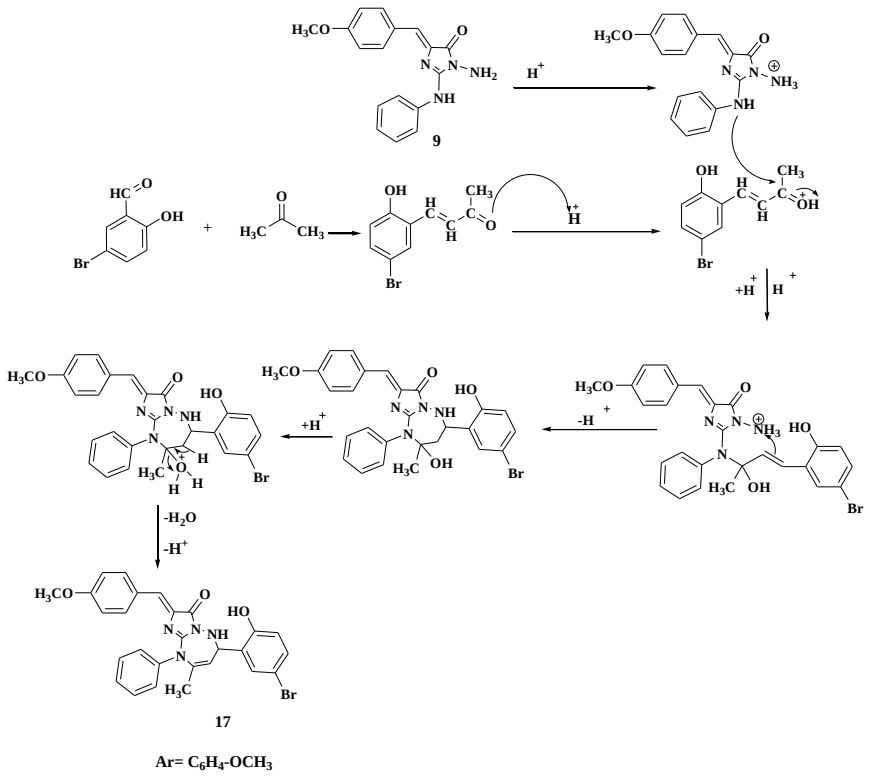
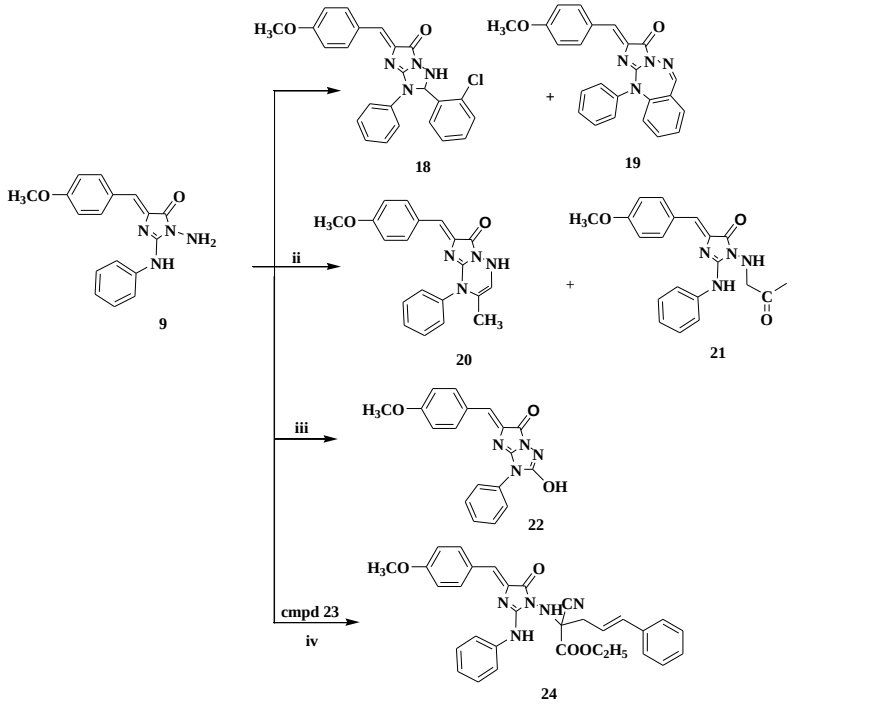


Figure 2. Postulated reaction mechanism for the synthesis of compound **17.**



Reagents and conditions: (i) Orthochlorobenzaldehyde/ dry benzene/ TEA, reflux;**(ii)** chloroacetone/ $NaOC_2H_5$/ refluX; **(iii)** Ethyl chloroformate/ $NaOC_2H_5$/ reflux; **(iv)** Ethyl 2-cyano-5-phenylpenta-2,4-dienoate **23**/ absolute ethanol/ Pip./ reflux.

Scheme IV

Table 1. The mean growth inhibition percent, delta values and the percent growth inhibition against some subpanel cell lines of selected compounds of scheme II & III.

Comp. No. (NCI No.)	Mean growth percent (Delta)	Panel: Subpanel cell line (Growth inhibition percent)
10 (806578)	**100.31 (26.52)**	**Non-Small cell lung cancer:** EKVX (7.36), NCI-H226 (8.99), NCI-H23 (6.90), NCI-H522 (23.25). **Colon cancer:** HT29 (7.62). **CNS Cancer:** SF539 (5.59). **Melanoma:** SK-MEL-2 (8.67), UACC-62 (7.08). **Ovarian Cancer:** IGROV1 (16.02). **Renal Cancer:** CAKI-1 (9.27), UO-31 (26.21). **Prostate Cancer:** PC-3 (6.44). **Breast Cancer:** MDA-MB-231/ATCC (7.75), HS 578T (9.36).
11 (806579)	**96.65 (27.65)**	**Non-Small cell lung cancer:** EKVX (27.18), HOP-92 (9.95), NCI-H226 (7.28), NCI-H23 (7.07). **CNS Cancer:** SF-539 (10.53), SNB-19 (12.54), U251 (9.30). **Melanoma**: SK-MEL-5 (7.28), UACC-62 (7.18). **Ovarian cancer:** IGROV1 (16.12). **Renal Cancer:** A498 (10.74), CAKI-1 (7.53), SN12C (7.57), UO-31 (28.77). **Breast Cancer:** MDA-MB-231/ATCC (8.98), HS 578T (31).
12 (806581)	**101.17 (23.74)**	**Non-Small cell lung cancer:** NCI-H226 (6.85), NCI-H23 (13.55). **Melanoma:** LOXIMVI (12.19). **Renal Cancer:** CAKI-1 (11.63), RXF 393 (10.28), UO-31 (22.57). **Breast Cancer:** MDA-MB-468 (11.09).
13 (806586)	**94.93 (37.65)**	**Non-Small cell lung cancer:** EKVX (31.41), HOP-92 (24.07), NCI-H522 (14.23). **CNS cancer:** SNB-19 (13.84), U251 (10.53). **Melanoma**: UACC-62 (12.71). **Ovarian Cancer:** IGROV1 (17.77), OVCAR-8 (7.56). **Renal Cancer:** 786-0 (11.55), A498 (27.51), UO-31 (27.12). **Breast Cancer:** MCF7 (9.66), MDA-MB-231/ATCC (13.71), HS 578T (42.72).
16 (806584)	**93.39 (67.50)**	**Non-Small cell lung cancer:** EKVX (51.33), HOP-92 (17.72), NCI-H226 (18.59). **CNS Cancer:** SF-268 (12.36). **Melanoma:** SK-MEL-5 (17.59), UACC-62 (56.07). **Ovarian Cancer:** IGROV1 (15.38), OVCAR-5 (10.74). **Renal Cancer:** A498 (19.62), SN-12C (14.45), UO-31 (20.05). **Prostate Cancer:** DU-145 (12.81). **Breast Cancer:** MDA-MB-231/ATCC (11.40), HS 578T (74.11).
17 (806585)	**97.40 (15.84)**	**Leukemia:** RPMI-8226 (8.52). **Non-Small cell lung cancer:** NCI-H226 (14.94). **Colon cancer:** HCC-2998 (12.9). **CNS cancer:** SNB-19 (6.24), U251 (6.21). **Melanoma**: MALME-3M (7.77), UACC-62 (9.38). **Ovarian Cancer:** IGROV1 (9.03), NCI/ADR-RES (6.10). **Renal Cancer:** UO-31 (18.44). **Prostate Cancer:** PC-3 (7.91). **Breast Cancer:** MCF7 (16.34), MDA-MB-231/ATCC (7.16), BT-549 (11.98), T-47D (10.39), MDA-MB-468 (9.31).

Table 2. The mean growth percent, delta values and the percent growth inhibition against some subpanel cell lines of selected compounds of scheme IV.

Comp. No. (NCI No.)	Mean growth percent (Delta)	Panel: Subpanel cell line (Growth inhibition percent)
18 (806589)	**98.64 (21.24)**	**Leukemia:** CCRF-CEM (18.04). **Non-Small cell lung cancer:** HOP-92 (9.30), NCI-H226 (8.38), NCI-H23 (8.75). **CNS Cancer:** SNB-75 (7.29). **Melanoma:** LOXIMVI (20.58). **Ovarian Cancer:** IGROV1 (8.93). **Renal Cancer:** A498 (10.60), CAKI-1 (12.31), RXF 393 (9.16), UO-31 (22.40). **Breast Cancer:** MDA-MB-231/ATCC (10.56), HS 578T (8.55), MDA –MB-468 (16.79).
19 (806590)	**98.39 (37.69)**	**Non-Small cell lung cancer:** EKVX (34.01). **Melanoma:** UACC-257 (9.73). **Ovarian cancer:** IGROV1 (8.93). **Renal Cancer:** CAKI-1 (15.64), UO-31 (22.82). **Prostate Cancer:** PC-3 (9.15). **Breast Cancer:** MDA-MB-231/ATCC (12.19), HS 578T (39.30).
20 (806580)	**99.36 (54.80)**	**Non-Small cell lung cancer:** EKVX (42.24), NCI-H226 (12.84). **Melanoma:** UACC-257 (20.86). **Renal Cancer:** UO-31 (10.79). **Breast Cancer:** HS 578T (55.44), BT-549 (14.67).
22 (806588)	**94.55 (28.94)**	**Non-Small cell lung cancer:** EKVX (34.39), HOP-92 (21.94), NCI-H226 (18.02). **Colon cancer:** HCT-15 (6.39). **CNS Cancer:** SF-268 (8.52), SNB-19 (11.82), U251 (12.80). **Melanoma:** MALME-3M (7.08), UACC-62 (8.35). **Ovarian Cancer:** IGROV1 (18.04), OVCAR-4 (8.87). **Renal Cancer:** 786-0 (10.90), A498 (24.59), CAKI-1 (8.36), RXF 393 (10.27), SN12C (7.49), UO-31 (23.82). **Breast Cancer:** MCF7 (12.24), HS 578T (30.34), BT-549 (11.29), MDA-MB-468 (12.33).
24 (806582)	**92.54 (64.34)**	**Non-Small cell lung cancer:** EKVX (64.05), HOP-62(9.01), NCI-H226 (17.41), **Colon Cancer:** NCI-H23 (16.07). **CNS Cancer:** SF-268 (11.32). **Melanoma:** SK-MEL-5 (18.86), UACC-62 (58.47). **Ovarian Cancer:** IGROV1 (41.99), SK-OV-3 (11.37). **Renal Cancer:** ACHN (11.48), CAKI-1 (19.32), SN-12C (17.55), UO-31 (31.98). **Breast Cancer:** MCF7 (13.77), MDA-MB-231/ATCC (25.24), HS 578T (71.80), BT-549 (11.95).

The postulated reaction mechanism for the synthesis of compound **17** is illustrated as follows:

The reaction of compound **9** with 2-chlorobenzaldehyde yielded a mixture of imidazotriazole derivative **18** and the imidazotriazepine derivative **19 "SchemeIV"**. **^{1}H NMR** spectrum of compound **18** showed a singlet signal at δ 3.65 ppm due to imidazotriazole C_2 proton and singlet signal at δ 6.55 ppm due to triazole-NH proton which disappeared by deterioration. While, **^{1}H NMR** spectrum of compound **19** revealed a singlet signal at δ 7.24 ppm due to triazepine C_3 proton. Also, compound **9** was reacted with chloroacetone give the imidazotriazine derivative **20** as well as the open chain derivative **21**. **^{1}H NMR** spectrum of compound **21** revealed two deuterium oxide exchangeable singlet signals at δ 7.28 and δ 11.78 ppm assignable to NH and NH-phenyl protons, respectively. The imidazotriazole derivative **22** was prepared via the reaction of compound **9** with ethyl chloroformate which its **^{1}H NMR** spectrum revealed a deuterium oxide

exchangeable singlet signal at δ 11.62 ppm due to hydroxyl proton. Finally, compound **9** was reacted with ethyl 2-cyano-5-phenylpenta-2,4-dienoate **23** [14] in absolute ethanol containing piperidine to yield the target compound **24**. **^{1}H NMR** spectrum of compound **24** displayed two multiplet signals at 1.45-1.63 ppm and 3.66-3.86 ppm attributed to methyl and methylene ester protons. Beside to, two deuterium oxide exchangeable singlet signals at δ 3.37 and 6.56 ppm corresponding to N-NH and NH-phenyl protons, respectively.

Part 2- Biology:

National Cancer Institute (NCI), USA selected eleven of the novel compounds, to be evaluated in a single high dose (10 µmole) for their anticancer activities.

As revealed from the results presented in **Tables 1 & 2,** that structural modifications to our lead compound Leucettamine B via fusion of nitrogen-containing five, six and seven membered rings to the imidazole nucleus at [1,2-b] junction. It can be noted that, fusion of triazole, pyridine, pyrimidine, thienopyrimidine and benzotetrazepine resulted in compounds with weak to mild activity against most cancer cell lines. However, the fusion of 3,5-diamino-6-phenyltetrazepine as in compound **16** and 4-phenyl-5-methyl[1,2,4]triazine as in compound **20** resulted in strong activity against Non-Small Cell Lung cancer EKVX, Melanoma UACC-62 and Breast cancer HS 578T cell lines. On the other hand, retaining the imidazole nucleus not fused and substituting N1 amino function with ethyl phenylpentenoate derivative as in compound **24** led to potent anticancer activity against the three aforementioned cell lines.

CONCLUSION

Conclusively, an effective approach for the synthesis of diversly functionalized anticancer analogs were established with high yield. Eleven of the newly synthesized compounds were selected by the National Cancer Institute, Bethesda, Maryland, USA, to be evaluated for their anticancer activities It can be concluded that, 8-(4-Methoxybenzylidene)-2,4-diamino-5-phenyl-5H-imidazo[1,2-b][1,2,4,6] tetrazepin-7(8H)-one; **16**, 6-(4-Methoxybenzylidene)-3-methyl-4-phenylimidazo[1,2-b][1,2,4]triazin-7(1H,4H,6H)-one; **20** and 4-Ethyl 2-(4-(4-methoxybenzylidene)-4,5-dihydro-5-oxo-2-(phenylamino)imidazol-1-ylamino)-2-cyano-5-phenylpent-4-enoate; **24** were found to possess selective anticancer activity against Non Small Cell Lung cancer EKVX, Melanoma UACC-62 and Breast cancer HS 578T cell lines.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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