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USES OF β-DIKETONES FOR THE SYNTHESIS OF NOVEL HETEROCYCLIC COMPOUNDS AND THEIR ANTITUMOR EVALUATIONS

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ABSTRACT. The reaction of the 3-oxo-N,3-diphenylpropan-amide (**3**) with either malononitrile or ethyl cyanoacetate in ammonium acetate gave the 1,2-dihydropyridine derivatives **6a** or **6b**, respectively. On the other hand, carrying the same reaction in the presence of triethylamine gave the 1,6-dihydropyridine derivatives **7a** and **7b**, respectively. Moreover, compound **3** reacted with 2-aminoprop-1-ene-1,1,3-tricarbonitrile to give the pyridine derivative **9**. Compound **7b** reacted with the active methylene derivatives **10a,b** and **4a,b** to give the naphthyridine derivatives **11a,b** and **12a,b**; respectively. Compound **3** was also used for the synthesis of thiophene derivatives **13a,b** and **16a,b**. In addition, the reaction of ethyl benzoylacetate (**1**) with *o*-phenylene diamine gave the benzimidazole derivative **18**. The reactivity of the latter product towards different reagents was studied to give different products. The cytotoxicity of the newly synthesized products was studied towards some cancer and normal cell lines, in addition toxicity of compounds was measured and docking of the most active compounds was done. Compounds **6b**, **7b**, **9**, **13a**, **13b**, **16a**, **20b**, **20c**, **24b**, **25** and **26b** exhibited optimal cytotoxic effect against cancer tested cell lines. These active compounds were evaluated against c-Met kinase using foretinib as the reference drug where all compounds expressed higher activity than the reference drug.

KEY WORDS: Ethyl benzoylacetate, Pyridine, Benzimidazole, Cytotoxicity

INTRODUCTION

It is of great interest to note that pyridine and pyrimidine derivatives were one of the most classes of compounds due to their high pharmaceutical and biological values. Especially fused pyridine derivatives have varieties of biological uses. Among such activities, they are known to exhibit high pharmacological, CNS depressant [1, 2], neuroleptic [3] and tuberculostatic [4] activities. In addition, a largenumber of pyridines were used as antimicrobial agents [5], inhibitors of glycogen syntheses kinase-3 (GSK-3) [6] and potent antitumor agents [7]. Moreover, benzimidazoles represent a group of compounds with a large number of pharmaceutical applications and many of them were basic nucleus of drugs structures. Some of them were naturally occurring nucleotides that capable to interact with biopolymers to enhance better biological properties. It was noticed that some 2-aminobenzimidazoles showed interesting antimicrobial effect, especially, their corresponding carbamate derivatives were obtained in good yields and exhibited significant *in vivo* antifilarial activity [8]. Within the field of drug designing such group of compounds have a great affinity towards different enzymes and protein receptors [9]. Optimization of benzimidazole-based structures has resulted in marketed drugs, e.g. within the field of chemotherapy it was found that omeprazole [10] and pimobendan [11] they were found not only as good therapeutically active drugs but also for treatment of heart feeler problems. Moreover, a large number of benzimidazoles derivatives are well known for their antimicrobial [12-15], anthelmintic [16], antiviral [17-18] and antifungal [19-21] activities. Many benzimidazole containing compounds are known as anticancer agents [22-28]. Within the field of topoisomerase inhibitors it was found that some benzimidazole was used as

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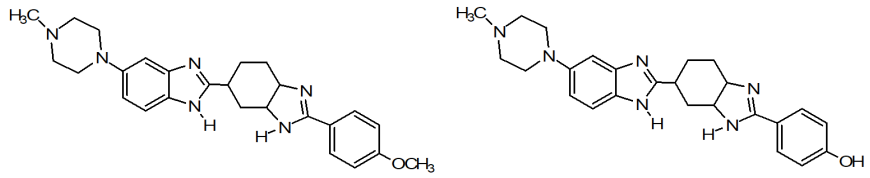
topoisomerase inhibitory drugs, e.g. Hoechst 33258 and Hoechst 33342 (Figure 1) [29, 30]. Within the field of DNA binders, it was also found that bis-benzimidazole derivatiives are used as head to head binders [31]. Within the field of cancer chemotherapy, many drugs containing benzimidazole nucleus are known through the market like RAF265 (CHIR-265; Novartis Pharmaceuticals, Basel, Switzerland) and AZD6244 (ARRY-142886; AstraZeneca, London, England). A very active known drug is RAF265 resulted in a reduction in tumor cell growth and in tumor cell apoptosis [32].



Hoechst 33342 **Hoechst 33258**

Figure 1. Examples of topoisomerase inhibitors containing benzimidazole nucleus.

Due to such high importance of pyridine and benzimidazole derivatives was focused on the efficient synthesis of new pyridine and benzimidazole derivatives starting from ethyl benzoylacetate followed by their cytotoxic evaluations against human cancer and normal cell lines.

EXPERIMENTAL

^{13}C NMR and ^{1}H NMR spectra were recorded on Bruker DPX200 instrument in $CDCl_3$ and DMSO with TMS as internal standard for protons and solvent signals as internal standard for carbon spectra. Chemical shift values are mentioned in δ (ppm). Mass spectra were recorded on EIMS (Shimadzu) and ESI-esquire 3000 Bruker Daltonics instrument. Elemental analyses were carried out by the Microanalytical Data Unit at Ludwig-Maximilians-Universität-München, Germany. The progress of all reactions was monitored by TLC on 2 x 5 cm pre-coated silica gel 60 F254 plates of thickness of 0.25 mm (Merck).

General procedure for the synthesis of the 1,2-dihydropyridine derivatives ***6a,b***

Either of malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) together with ammonium acetate (0.50 g) were added to a dry solid of compound **3** (2.16 g, 0.01 mol). The reaction mixture, in each case, was heated in an oil bath at 120 °C for 15 min. The solid product formed after boiling with ethanol was collected by filtration.

*6-Hydroxy-2-imino-1,4-diphenyl-1,2-dihydropyridine-3-carbonitrile (**6a**)*. Yellow crystals (EtOH), yield 74% (2.12 g), mp 119-121 °C. IR ν_{max} cm^{-1}: 3544-3260, 3056, 2957, 2207, 1688, 1662, 1604 (C=C). ^{1}H NMR (DMSO-d_6 400 MHz): δ = 4.15 (s, 2H, CH_2), 7.30-7.99 (m, 10H, $2C_6H_5$), 10.21 (s, 1H, NH). ^{13}C NMR (DMSO-d_6 75 MHz): δ = 49.5 (pyridine C-3), 103.6, 104.5 (C=C), 119.5 (CN), 120.19, 121.3, 123.9, 124.1, 124.8, 125.2, 125.5, 126.0, 134.5, 136.8 (two benzene, pyridine C), 165.9 (C=O), 175.2 (C=N). EIMS m/z 287 $[M]^+$ (20); anal. calcd. for $C_{18}H_{13}N_3O$ (287.11): C, 75.25; H, 4.56; N, 14.63. Found: C, 72.06; H, 4.72; N, 14.88.

*Ethyl 6-hydroxy-2-imino-1,4-diphenyl-1,2-dihydropyridine-3-carboxylate (**6b**)*. Yellow crystals (EtOH), yield 70% (2.33 g), mp 277-279 °C. IR ν_{max} cm^{-1}: 3443-3259, 3092, 2959, 2888, 1695, 1689, 1636. ^{1}H NMR (DMSO-d_6 400 MHz): δ = 1.14 (t, 3H, J = 7.03 Hz, $OCH_2\underline{CH_3}$), 4,18 (q,

2H, J = 7.03 Hz, O<u>CH$_2$</u>CH$_3$), 5.95 (s, 2H, CH$_2$), 7.30-7.99 (m, 10H, 2C$_6$H$_5$), 10.21 (s, 1H, D$_2$O exchangeable, NH). ^{13}C NMR (DMSO-d$_6$ 75 MHz) δ: 13.6 (OCH$_2$<u>CH$_3$</u>), 50.3 (O<u>CH$_2$</u>CH$_3$), 50.6 (pyridine C-3), 103.8, 104.9 (C=C), 116.5 (CN), 119.0, 120.5, 122.5, 123.6, 124.4, 124.6, 125.1, 125.7 (two benzene C), 163.8 (C=N), 165.1, 166.4 (2C=O), EIMS m/z 334 [M]$^+$ (28). Anal. calcd. for C$_{20}$H$_{18}$N$_2$O$_3$ (334.38): C, 71.84; H, 5.43; N, 8.38 %. Found: C, 71.69; H, 5.51; N, 8.49.

General procedure for the synthesis of the 1,6-dihydopyridine-2-carboxylate ***7a,b***

Either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added to a solution of compound **3** (2.16 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture, in each case was heated under reflux for 3 h then evaporated under vacuum. The solid product produced after triturating the remaining product with diethyl ether was collected by filtration.

*4-Amino-5-benzoyl-6-oxo-1-phenyl-1,6-dihydropyridine-2-carbonitrile (**7a**)*. Pale yellow crystals (1,4-dioxane), yield 80% (2.12 g), mp 160-168 °C. IR ν$_{max}$ cm^{-1}: 3544-3260, 3058, 2957, 1705, 1689, 1632. ^{1}H NMR (DMSO-d$_6$ 400 MHz): δ = 4.28, 5.31 (2s, 4H, D$_2$O exchangeable, 2NH$_2$), 6.15 (s, 1H, pyridine H-4), 7.31-7.68 (m, 10H, 2C$_6$H$_5$). ^{13}C NMR (DMSO-d$_6$ 75 MHz): δ = 119.5, 120.1, 120.7, 122.0, 123.6, 124.7, 125.6, 127.3, 129.8, 130.2, 131.6, 139.4 (two benzene, pyridine C), 164.3, 166.4 (2 C=O). EIMS m/z 305 [M]$^+$ (26). Anal. calcd. for C$_{18}$H$_{15}$N$_3$O$_2$ (305.54): C, 70.81; H, 4.95; N, 13.76.

*Ethyl 4-amino-5-benzoyl-6-oxo-1-phenyl-1,6-dihydropyridine-2-carboxylate (**7b**)*. Yellow crystals (1,4-dioxane), yield 81% (2.47 g), mp 177-179 °C. IR ν$_{max}$ cm^{-1}: 3540-3320, 3065, 2955, 2889, 1718, 1688, 1633. ^{1}H NMR (DMSO-d$_6$ 400 MHz): δ = 4.25 (s, 2H, D$_2$O exchangeable, NH$_2$), 6.11 (s, 1H, pyridine H-4), 7.28-7.67 (m, 10H, 2C$_6$H$_5$), 11.42 (s, 1H, D$_2$O exchangeable, OH). ^{13}C NMR (DMSO-d$_6$ 75 MHz): δ = 119.6, 120.2, 120.9, 122.5, 123.8, 124.3, 124.6, 125.7, 129.8, 130.2, 133.2, 139.4 (two benzene, pyridine C), 164.2, 166.8 (2 C=O). EIMS m/z 306.32 [M]$^+$ (28). Anal. calcd. for C$_{18}$H$_{14}$N$_2$O$_3$ (306.32): C, 70.58; H, 4.61; N, 9.15. Found: C, 70.80; H, 4.88; N, 9.05.

*2,4-Diamino-7-oxo-5,8-diphenyl-7,8-dihydro-1,8-naphthyridine-3-carbonitrile (**9**)*

2-Aminoprop-1-ene-1,1,3-tricarbonitrile (**8**) (1.32 g, 0.01 mol) was added to a solution of compound **3** (2.16 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 6 h then evaporated under vacuum. The solid product produced after triturating the remaining product with diethyl ether was collected by filtration. Pale yellow crystals (1,4-dioxane), yield 86% (3.03 g), mp 260-263 °C. IR ν$_{max}$ cm^{-1}: 3473, 3327, 3056, 2220, 1688, 1623. ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ = 4.92, 5.68 (2s, 4H, D$_2$O exchangeable, 2NH$_2$), 6.80 (s, 1H pyridine H-6), 7.31-7.58 (m, 10H, 2C$_6$H$_5$). ^{13}C NMR (DMSO-d$_6$, 75 MHz): δ = 116.8 (CN), 120.1, 121.3, 122.3, 123.5, 124.0, 124.8, 125.1, 126.4, 128.1, 130.2, 131.2, 131.8, 133.2, 134.2 (two C$_6$H$_5$, two pyridine C), 162.4 (C=O), 169.5 (C=N). EIMS m/z 353 [M]$^+$ (22). Anal. calcd. for C$_{21}$H$_{15}$N$_3$O (353.39): C, 71.38; H, 4.28; N, 19.82. Found: C, 71.42; H, 4.31; N, 19.72.

General procedure for the synthesis of the 1,6-naphthridine derivatives ***11a,b***

Either acetylacetone (1.00 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) was added to a solution of compound **7b** (3.06 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 4 h then poured onto ice/water and the formed solid product was collected by filtration.

8-Benzoyl-2,4-dimethyl-6-phenyl-1,6-naphthridine-5,7(1H,6H)-dione ***(11a)***. Orange crystals (1,4-dioxane) yield 76% (2.81 g), mp 116-118 °C. IR ν_{max} cm^{-1}: 3430-3261, 3065, 2919, 1688-1662. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 2.51, 4.1 (2s, 6H, $2CH_3$), 5.95 (s, 1H, pyridine H-3), 7.25-7.54 (m, 10H, $2C_6H_5$), 10.16 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 39.6, 49.8 (2 CH_3), 119.9, 120.0, 123.9, 124.2, 124.8, 125.8, 126.0, 126.3, 129.3.2, 129.4, 130.6, 132.6, 134.1, 134.2 (two benzene C, two pyridine C), 165.8, 170.2, 175.2 (3 C=O). EIMS m/z 370 $[M]^+$ (20). Anal. calcd. for $C_{23}H_{18}N_2O_3$ (370.41): C, 74.58; H, 4.90; N, 7.56. Found: C, 74.66; H, 4.69; N, 7.38.

8-Benzoyl-4-hydroxy-2-methyl-6-phenyl-1,6-naphthridine-5,7(1H,6H)-dione ***(11b)***. Yellow crystals (1,4-dioxane), yield 80% (2.97 g), mp 97-99 °C. IR ν_{max} cm^{-1}: 3550-3320, 3055, 2955, 2889, 1720-1688, 1633. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 2.51 (s, 3H, CH_3), 5.45 (s, 1H, pyridine H-3), 7.01-7.31 (m, 10H, $2C_6H_5$), 8.20 (s, 1H, D_2O exchangeable, NH), 9.01 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 25.1, (CH_3), 118.9, 119.8, 120.3, 123.6, 123.9, 124.2, 125.3, 125.6, 128.6, 129.4, 132.1, 133.6, 134.2, 134.6 (two benzene, two pyridine C), 165.6, 172.0, 180.3 (3 C=O). EIMS m/z 372 $[M]^+$ (18). Anal. calcd. for $C_{22}H_{16}N_2O_4$ (372.38): C, 70.96; H, 4.33; N, 7.52. Found: C, 70.82; H, 4.48; N, 7.63.

General procedure for the synthesis of the 5,6,7,8-tetrahydro-1,6-naphthyridine derivatives ***12a,b***

Either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added to a solution of compound **7b** (3.06 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 8 h then poured onto ice/water and the formed solid product was collected by filtration.

2-Amino-5,7-dioxo-4,6-diphenyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile ***(12a)***. Yellow crystals (1,4-dioxane), yield 88% (3.11 g), mp 140-143 °C. IR ν_{max} cm^{-1}: 3409-3334, 3055, 2923, 2210, 1680-1662. ^{1}H NMR (DMSO-d_6 400 MHz):δ = 3.58, 4.20 (2s, 4H, D_2O exchangeable, $2NH_2$), 6.20 (s, 1H, pyridine H-3), 7.20 (s, 1H, pyridine H-3), 7.27-7.54 (m, 10H, $2C_6H_5$). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 48.9 (pyridine C-3), 119.9, 120.3, 120.6, 123.9, 125.8, 128.6, 129.3.2, 129.4, 132.6, 134.1, 140.4 (two benzene C, two pyridine C), 165.8, 166.3, 170.1 (3 C=O), 195.3 (C=N). EIMS m/z 372$[M]^+$ (20); anal. calcd. for $C_{21}H_{16}N_4O_3$ (372.38): C, 67.73; H, 4.33; N, 15.05. Found: C, 67.58; H, 4.04; N, 15.25.

2-Hydroxy-5,7-dioxo-4,6-diphenyl-5,6,7,8-tetrahydro-1,6-naphthyridine-3-carbonitrile ***(12b)***. Yellow crystals (1,4-dioxane), yield 70% (2.48 g), mp 100-103 °C. IR ν_{max} cm^{-1}: 3542-3260, 3093, 2956, 1688-1662, 1605. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 3.88 (s, 2H, D_2O exchangeable, NH_2), 4.14 (s, 1H, pyridine H-3), 7.27-7.67 (m, 11H, $2C_6H_5$, pyridine H-3), 10.19 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6 75 MHz) δ = 49.5 (pyridine C-3), 119.8, 120.2, 122.5, 123.6, 124.2, 124.6, 125.3, 125.8, 130.2, 132.5, 133.2, 134.1, 134.6. 135.2 (two benzene C, two pyridine C), 165.6, 170.1 (2C=O), 171.1 (C=N). EIMS m/z 373 $[M]^+$ (20). Anal. calcd. for $C_{21}H_{15}N_3O_4$ (373.37): C, 67.56; H, 4.05; N, 11.25. Found: C, 67.33; H, 3.87; N, 11.41.

General procedure for the synthesis of the thiophene derivatives ***13a,b***

Each of elemental sulfur (0.32 g, 0.01 mol) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added to a solution of compound **3** (2.16 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture, in each case, was heated under reflux for 3 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration.

*5-Amino-4-cyano-N,3-diphenylthiophene-2-carboxamide (**13a**)*. Orange crystals (1,4-dioxane), yield 90% (2.87 g), mp 120-122 °C. IR ν_{max} cm^{-1}: 3439-33259, 3056, 2923, 2211, 1687. ^1H NMR (DMSO-d_6 400 MHz): δ = 4.15 (s, 2H, D_2O exchangeable, NH_2), 7.31-7.58 (m, 10H, $2C_6H_5$), 10.21 (s, 1H, D_2O exchangeable, NH), .^{13}C NMR (DMSO-d_6 75 MHz): δ = 117.5 (CN), 120.3, 121.6, 122.8, 123.6, 124.4, 124.8, 125.1, 125.8, 136.7, 139.5. 140.4, 142.2 (two benzene C, thiophene C), 165.8 (C=O). EIMS m/z 319 $[M]^+$ (26). Anal. calcd. for $C_{18}H_{13}N_3OS$ (319.38): C, 67.69; H, 4.10; N, 13.16; S, 10.04. Found: C, 67.56; H, 3.89; N, 12.88; S, 10.38.

*Ethyl 2-amino-4-phenyl-5-(phenylcarbamoyl)thiophene-3-carboxylate (**13b**)*. Orange crystals (1,4-dioxane), yield 73% (2.67 g), mp 90-93 °C. IR ν_{max} cm^{-1}: 3426-3261, 3056, 2955, 2890, 1687-1661, 1604 (C=C). ^1H NMR (DMSO-d_6 400 MHz) δ = 1.17 (t, 3H, *J* = 6.09 Hz, $OCH_2\underline{CH_3}$), 4.18 (q, 2H, *J* = 6.09 Hz, $O\underline{CH_2}CH_3$), 5.90 (s, 2H, D_2O exchangeable, NH_2), 7.31-7.99 (m, 10H, $2C_6H_5$), 10.01 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 16.5 ($OCH_2\underline{CH_3}$), 50.2 ($O\underline{CH_2}CH_3$), 119.4, 119.9, 121.9, 123.8, 124.6, 125.1, 125.5, 126.2, 132.3, 133.1, 134.6, 135.3 (two C_6H_5, thiophene C), 165.8, 167.1 (2C=O). EIMS m/z 366 $[M]^+$ (28). Anal. calcd. for $C_{20}H_{18}N_2O_3S$ (366.43): C, 65.55; H, 4.95; N, 7.64; S, 8.75. Found: C, 65.72; H, 4.73; N, 7.73; S, 8.93.

*General procedure for the synthesis of the 4-(phenylcarbamoyl)thiophene derivatives **16a,b***

To a solution of compound **2** (2.39 g, 0.01 mol) in dimethylformamide (30 mL) containing potassium hydroxide (0.56 g, 0.01 mol) phenylisothiocyanate was added. The reaction mixture was stirred at room temperature for 24 h then to the reaction mixture either ethyl chloroacetate (1.22 g, 0.01 mol) or (0.92 g, 0.01 mol) was added. The reaction mixture was stirred at room temperature for an additional 24 h then poured onto ice/water containing few drops of hydrochloric acid (till pH 6) and the formed solid product was collected by filtration.

*Ethyl 3-phenyl-5-(phenylamino)-4-(phenylcarbamoyl)thiophene-2-carboxylate (**16a**)*. Yellow crystals (1,4-dioxane), yield 86% (3.80 g), mp 160-163 °C. IR ν_{max} cm^{-1}: 3397-3229, 3048, 2986, 2890, 1688-1668, 1624. ^1H NMR (DMSO-d_6 400 MHz) δ = 1.21 (t, 3H, *J* = 7.21 Hz, $OCH_2\underline{CH_3}$), 3.87(q, 2H, J = 7.21 Hz, $\underline{OCH_2}CH_3$), 7.43-7.49 (m, 15H, $3C_6H_5$), 8.20, 10.39 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ = 16.6 ($OCH_2\underline{CH_3}$), 50.6 ($\underline{OCH_2}CH_3$), 120.0, 121.8, 122.6, 123.8, 123.9, 124.2, 124.5, 125.2, 125.3, 125.8, 126.3, 126.9, 130.4, 133.1, 138.0, 139.2 (three C_6H_5 and thiophene C), 165.8, 167.1 (2C=O). EIMS m/z 366 $[M]^+$ (28). Anal. calcd. for $C_{26}H_{22}N_2O_3S$ (442.53): C, 70.57; H, 5.01; N, 6.33; S, 7.24. Found: C, 70.29; H, 4.87; N, 6.58; S, 7.42.

*5-Methyl-N,4-diphenyl-2-(phenylamino)thiophene-3-carboxamide (**16b**)*. Orange crystals (1,4-dioxane), yield 79% (3.25 g), mp 175-177 °C. IR ν_{max} cm^{-1}: 3453-3422, 3054, 2932, 1720-1668. ^1H NMR (DMSO-d_6 400 MHz): δ = 3.56 (s, 3H, CH_3), 7.24-7.56 (m, 15H, $3C_6H_5$), 10.51, 11.30 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 16.6 (CH_3), 119.2, 120.5, 120.8, 121.2, 121.7, 122.5, 123.6, 123.8, 124.8, 125.3, 125.6, 126.2, 130.4, 132.5, 133.8, 134.1 (three benzene C, thiophene C), 167.8, 172.5 (2C=O). EIMS m/z 412 $[M]^+$ (20). Anal. calcd. for $C_{25}H_{20}N_2O_2S$ (412.51): C, 72.79; H, 4.89; N, 6.79; S, 7.77. Found: C, 72.95; H, 4.63; N, 6.56; S, 8.04.

*2-(1H-Benzo[d]imidazol-2-yl)-1-phenylethanone (**18**)*

Equimolar amounts of ethyl benzoylacetate (1.92 g, 0.01 mol) and o-phenylenediamine (1.09 g, 0.01 mol) were heated at 120 °C in the dry conditions in an oil bath for 10 min then the reaction mixture was left to cool. The formed solid product was triturated with diethylether then

collected by filtration. Pale yellow crystals (1,4-dioxane), yield 72% (1.69 g), mp 180-183 °C. IR ν_{max} cm^{-1}: 3437-3302, 3053, 2974, 1680, 1615. ^1H NMR (DMSO-d_6 400 MHz): δ = 3.50 (s, 2H, CH_2), 7.20-7.55 (m, 9H, C_6H_5, C_6H_4), 10.45 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (DMSO-d_6 75 MHz): δ = 49.8 (CH_2), 118.2, 123.8, 124.9, 125.0, 125.3, 126.0, 126.4, 126.8, 128.3, 129.6 (benzene, indole C), 164.8 (C=O), 167.2 (C=N). EIMS m/z 236 $[M]^+$ (90). Anal. calcd. for $C_{15}H_{12}N_2O$ (236.27): C, 76.25; H, 5.12; N, 11.86. Found: C, 76.39; H, 4.82; N, 11.66.

General procedure for the synthesis of the benzo[d]imidazol derivatives ***20a-c***

Either benzaldehyde (1.06 g, 0.01 mol) 4-methoxybanzaldehyde (1.36 g, 0.01 mol) or 4-chlorobenzaldehyde (1.40 g, 0.01 mol) was added to a solution of compound **18** (2.36 g, 0.01 mol) in 1,4-dioxane (40 mL) containing piperidine (0.50 mL). The reaction mixture, in each case, was heated under reflux for 3 h then poured onto ice/water containing few drops of hydrochloric acid. The formed oily product was triturated with ethanol and the precipitated product was collected by filtration.

2-(1H-Benzo[d]imidazol-2-yl)-1,3-diphenylprop-2-en-1-one ***(20a)***. Pale yellow crystals (1,4-dioxane), yield 68% (2.20 g), mp 183-187 °C. IR ν_{max} cm^{-1}: 3537-3425, 3053, 1680, 1615. ^1H NMR (DMSO-d_6 400 MHz): δ = 6.06 (s, 1H, CH), 7.26-7.55 (m, 14H, $2C_6H_5$, C_6H_4), 10.54 (s, 1H, D_2O exchangeable NH). ^{13}C NMR (DMSO-$d_6$75 MHz) δ: 85.1, 112.3 (C=C), 120.3, 122.2, 122.6, 123.1, 123.5, 124.0, 124.6, 125.0, 125.4, 125.7, 127.5, 127.3 ($2C_6H_5$, C_6H_4), 160.2 (CO), 173.4 (C=N). EIMS m/z 324$[M]^+$ (48); anal. calcd. for $C_{22}H_{16}N_2O$ (324.38): C, 81.46; H, 4.97; N, 8.64. Found: C, 81.66; H, 5.26; N, 8.47.

2-(1H-Benzo[d]imidazol-2-yl)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one ***(20b)***. Orange crystals (1,4-dioxane), yield 78% (2.76 g), mp 145-147 °C. IR ν_{max} cm^{-1}: 3424-3244, 3058, 1710, 1666. ^1H NMR (DMSO-d_6 400 MHz) δ = 3.50 (s, 3H, CH_3), 6.08 (s, 1H, CH), 7.26-7.55 (m, 13H, C_6H_5, $2C_6H_4$), 10.54 (s, 1H, D_2O exchangeable NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 38.7 (CH_3), 89.2, 113.8 (C=C), 120.1, 122.4, 122.7, 123.0, 123.3, 124.1, 124.5, 125.2, 125.8, 125.3, 127.1, 127.2 (C_6H_5. $2C_6H_4$), 161.2 (CO), 172.8 (C=N). EIMS m/z 354 $[M]^+$ (25). Anal. calcd. for $C_{23}H_{18}N_2O_2$ (354.41): C, 77.95; H, 5.12; N, 7.90. Found: C, 78.28; H, 5.08; N, 8.21.

2-(1H-Benzo[d]imidazol-2-yl)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-one ***(20c)***. Yellow crystals (1,4-dioxane), yield 72% (2.57 g), mp 160-163 °C. IR ν_{max} cm^{-1}: 3423-3294, 3055, 1720, 1660. ^1H NMR (DMSO-d_6 400 MHz) δ = 6.08 (s, 1H, CH), 7.26-7.55 (m, 13H, C_6H_5, $2C_6H_4$), 10.54 (s, 1H, D_2O exchangeable NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ = 87.3, 112.6 (C=C), 119.2, 122.4, 122.9, 123.0, 123.3, 124.2, 124.8, 125.3, 125.9, 126.1, 127.7, 128.6 (C_6H_5. $2C_6H_4$), 161.4 (CO), 171.3 (C=N). EIMS m/z 358 $[M]^+$ (38); anal. calcd. for $C_{22}H_{15}ClN_2O$ (358.83): C, 73.64; H, 4.21; N, 7.81. Found: C, 73.75; H, 4.52; N, 7.95.

2-(3,5-Diphenyl-1H-pyrazol-4-yl)-1H-benzo[d]imidazole ***(21)***

To a solution of compound **20a** (3.24 g, 0.01 mol) in 1,4-dioxane (40 mL) hydrazine hydrate (0.50 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h then poured onto ice/water containing few drops of hydrochloric acid and the formed solid product was collected by filtration. Yellow crystals (EtOH), yield 68% (2.28 g), mp 221-223 °C. IR ν_{max} cm^{-1}: 3440-3302, 3054, 1662, 1608. ^1H NMR (DMSO-d_6 400 MHz) δ = 7.28-7.74 (m, 14H, $2C_6H_5$, C_6H_4), 10.12, 11.20 (2s, 2H, D_2O exchangeable, 2NH), ^{13}C NMR (DMSO-d_6 75 MHz) δ: 118.3, 121.5, 121.9, 122.6, 123.8, 124.3, 124.5, 125.8, 126.0, 126.2, 127.6, 130.6, 139.2, 140.4 ($2C_6H_5$, C_6H_4, pyrazole C), 163.4, 170.0 (two C=N). EIMS m/z 336 $[M]^+$ (66); anal. calcd. for $C_{22}H_{16}N_4$ (336.40): C, 78.55; H, 4.79; N, 16.66. Found: C, 78.39; H, 4.86; N, 16.41.

*1-Amino-4-benzoyl-3-(4-methoxyphenyl)benzo[4,5]imidazo[1,2-a]pyridine-2-carbonitrile (**22**)*

Malononitrile (0.66 g, 0.01 mol) was added to a solution of compound **20b** (3.54 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 4 h then evaporated under vacuum. The remaining product was triturated with ethanol and the formed solid product was collected by filtration. Yellow crystals (EtOH), yield 73% (3.05 g), mp 182-185 °C. IR ν_{max} cm^{-1}: 3438-3304, 3054, 2200, 1687, 1662, 1614. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 3.50 (s, 3H, OCH_3), 5.02 (s, 2H, D_2O exchangeable, NH_2), 7.21-7.55 (m, 13H, C_6H_5, $2C_6H_4$), ^{13}C NMR (DMSO-d_6 75 MHz) δ: 46.8 (OCH_3), 116.9 (CN), 120.3, 120.6, 121.5, 121.8, 122.3, 123.2, 123.7, 124.7, 124.8, 125.0, 125.5, 126.7, 130.4, 131.6, 137.7, 139.9 (C_6H_5, $2C_6H_4$, pyridine C), 163.2 (C=O), 166.8 (C=N). EIMS m/z 418 $[M]^+$ (48); anal. calcd. for $C_{26}H_{18}N_4O_2$ (418.54): C, 74.63; H, 4.34; N, 13.39. Found: C, 74.49; H, 4.62; N, 13.61.

*General procedure for the synthesis of the arylhydrazone derivatives **24a,b***

To a cold solution (0-5 °C) of compound **18** (2.36 g, 0.01 mol) in ethanol (50 mL) containing sodium acetate (3.50 g, 0.50 mol) either benzenediazonium chloride (0.01 mol) or 4-chloro-benzenediazonium chloride (0.01 mol [prepared by adding a cold solution of sodium nitrite (0.70 g, in water (10 mL)) to a cold solution (0-5 °C) of either aniline (0.93 g, 0.01 mol) or 4-chloroaniline (1.27 g, 0.01 mol) in concentrated hydrochloric acid (12 mL) with continuous stirring] was added with continuous stirring. The whole reaction mixture was left at room temperature for 1 h then, the formed solid product was collected by filtration.

*2-(1H-Benzo[d]imidazol-2-yl)-1-phenyl-2-(2-phenylhydrazono)ethanone (**24a**)*. Orange crystals (EtOH), yield 88% (2.99 g), mp 167-169 °C. IR ν_{max} cm^{-1}: 3440-3299, 3053, 1668, 1610. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 7.26-7.55 (m, 14H, $2C_6H_5$, C_6H_4), 8.05, 10.55 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 121.0, 121.4, 122.6, 123.1, 123.5, 124.2, 124.6, 125.0, 125.3, 126.0, 128,2, 128.4, 130.4, 130.9, 137.7, 139.9 ($2C_6H_5$, C_6H_4), 163.0 (C=O), 166.8, 168.4 (2C=N). EIMS m/z 340 $[M]^+$ (28); anal. calcd. for $C_{21}H_{16}N_4O$(340.13): C, 74.10; H, 4.74; N, 16.46. Found: C, 74.33; H, 4.59; N, 16.79.

*2-(1H-Benzo[d]imidazol-2-yl)-1-phenyl-2-(2-(4-chlorophenylhydrazono)ethanone (**24b**)*. Orange crystals from (EtOH), yield 80% (2.99 g), mp 155-157 °C. IR ν_{max} cm^{-1}: 3442-3302, 3055, 1666, 1610. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 7.26-7.58 (m, 13H, C_6H_5, $2C_6H_4$), 8.08, 10.55 (2s, 2H, D_2O exchangeable, 2NH). ^{13}C NMR (DMSO-d_6 75 MHz) δ: 120.4, 121.3, 122.7, 123.3, 123.8, 124.0, 124.4, 125.1, 125.5, 126.4, 128.1, 128.5, 130.7, 130.4, 138.2, 139.5 (C_6H_5, $2C_6H_4$), 163.2 (C=O), 166.8, 168.6 (2C=N). EIMS m/z 374 $[M]^+$ (56). Anal. calcd. for $C_{21}H_{15}N_4O$ (374.83): C, 67.29; H, 4.03; N, 14.95. Found: C, 67.51; H, 3.86; N, 15.04.

*(1,3-Diaminobenzo[4,5]imidazo[1,2-a]pyridin-4-yl)(phenyl)methanone (**25**)*

Malononitrile (0.66 g, 0.01 mol) was added to a solution of compound **18** (2.36 g, 0.01 mol) or **3b** (2.30 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 4 h then poured onto ice/water and the formed solid product was collected by filtration. Yellow crystals (EtOH), yield 75% (2.26 g), mp 190-193 °C. IR ν_{max} cm^{-1}: 3426-3201, 3055, 1670, 1570. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 4.10, 5.40 (2s, 4H, D_2O exchangeable, $2NH_2$), 7.26-7.55 (m, 10H, C_6H_5, C_6H_4, pyridine H-3). ^{13}C NMR (DMSO-d_6 75 MHz) δ = 116.2, 120.3, 120.4, 121.1, 122.3, 122.7, 123.5, 123.9, 124.8, 128.1, 128.6, 130.4, 142,3, 142.6, 152.6 (C_6H_5, C_6H_4, pyridine C), 165.7 (C=O), 171.3 (C=N). EIMS: m/z = 302 $[M]^+$ (24); anal. calcd. for $C_{18}H_{14}N_4O$ (302.34): C, 71.51; H, 4.67; N, 18.53. Found: C, 71.39; H, 4.48; N, 18.71.

General procedure for the synthesis of the benzo[4,5]imidazo[1,2-a]pyridine derivatives ***26a,b***

Either acetylacetone (1.0 g, 0.01 mol) or ethyl acetoacetate (1.30 g, 0.01 mol) to a solution of compound **18** (2.36 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL). The reaction mixture was heated under reflux for 2 h then poured onto ice/water and the formed solid product was collected by filtration.

*(1,3-Dimethylbenzo[4,5]imidazo[1,2-a]pyridine-4-yl)(phenyl)methanone (**26a**)*. Yellow crystals (EtOH), yield 68% (2.10 g), mp 130-133 °C. IR ν_{max} cm^{-1}: 3058, 1720, 1620. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 2.42, 3.34 (2s, 6H, $2CH_3$), 6.61 (s, 1H, pyridine H-3), 7.27-7.36 (m, 9H, C_6H_5, C_6H_4). ^{13}C NMR (DMSO-d_6 75 MHz) δ = 24.3, 26.2 ($2CH_3$), 118.6, 121.4, 121.8, 123.5, 124.5, 125.3, 125.8, 126.1, 127,3, 129.2, 130.4, 140.5, 143.0, 150.3 (C_6H_5, C_6H_4, pyridine C), 165.5 (C=O), 167.2 (C=N). EIMS m/z 300 $[M]^+$ (68). Anal. calcd. for $C_{21}H_{16}N_4O$ (300.36): C, 79.98; H, 5.37; N, 9.33. Found: C, 79.69; H, 5.48; N, 9.59.

*1-Hydroxy-3-methylbenzo[4,5]imidazo[1,2-a]pyridine-4-yl)(phenyl)methanone (**26b**)*. Orange crystals (EtOH), yield 77% (2.32 g), mp 145-147 °C. IR ν_{max} cm^{-1}: 3435-3125, 3058, 1696, 1474. ^{1}H NMR (DMSO-d_6 400 MHz) δ = 3.34 (s, 3H, CH_3), 6.63 (s, 1H, pyridine H-3), 7.27-7.38 (m, 9H, C_6H_5, C_6H_4), 10.50 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (DMSO-d_6 75 MHz) δ = 24.1 (CH_3), 119.2, 122.0, 122.8, 123.1, 123.5, 124.7, 125.2, 126.3, 127.3, 129.2, 132.6, 142.1, 143.0, 144.6 (C_6H_5, C_6H_4, pyridine), 166.4 (C=O), 168.2 (C=N). EIMS m/z 302 $[M]^+$ (28); anal. calcd. for $C_{19}H_{14}N_2O_2$ (302.33): C, 75.48; H, 4.67; N, 9.27. Found: C, 75.21; H, 4.59; N, 9.41.

Chemicals

Chemical used through the anticancer evaluations of the synthesised compounds were obtained from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures

The cancer cell lines namely the human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). The cytotoxicity of the newly synthesised compounds against the six cancer cell lines and the normal fibroblast were demonstrated through Table 1.

c-Met enzymatic activity of the most active compounds

Using homogeneous time-resolved fluorescence (HTRF) assay as previously reported the c-Met kinase activities of compounds **6b**, **7b**, **9**, **13a**, **13b**, **16a**, **20b**, **20c**, **24b**, **25** and **26b** were evaluated. The IC_{50}'s were expressed through Table 2 using foretinib as the positive control. The data revealed that the three compounds expressed high enzymatic activity toward c-Met with IC_{50}'s much higher than that of the reference foretinib.

Table 1. Cytotoxicity of the newly synthesized compounds against a variety of cancer cell lines [IC_{50}[b] (nM)].

Compound	Cytotoxocity (IC50 in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
6a	2140	3228	2314	2230	3219	2240	na
6b	66	80	238	232	470	128	na
7a	2201	1488	2150	2068	3188	2146	na
7b	380	120	114	1340	666	1504	na
9	222	360	1128	2134	1240	1280	na
11a	2210	2395	1163	1446	1178	3430	na
11b	1320	1348	129	330	2177	2166	na
12a	3270	2870	2244	1252	1049	2271	na
12b	1282	1320	1160	870	1140	1160	na
13a	860	1028	759	1025	1254	1220	na
13b	750	539	1220	299	1186	1228	380
16a	182	268	560	2028	1130	328	na
16b	1135	1062	1011	1210	466	210	na
18	2220	2440	1128	2432	2143	2056	na
20a	1240	1562	1063	1260	1619	1655	na
20b	480	682	1158	1324	1460	1603	na
20c	330	268	98	64	1209	332	na
21	2262	2152	1381	1060	1313	1145	na
22	1080	150	840	324	360	1246	665
24a	1149	2160	3261	2136	2484	2868	na
24b	380	122	33	669	1240	890	na
25	487	350	3340	229	82	262	na
26a	1445	3110	3013	3076	670	2742	na
26b	32	48	260	82	71	559	na
CHS 828	25	2315	2067	1245	15	18	na

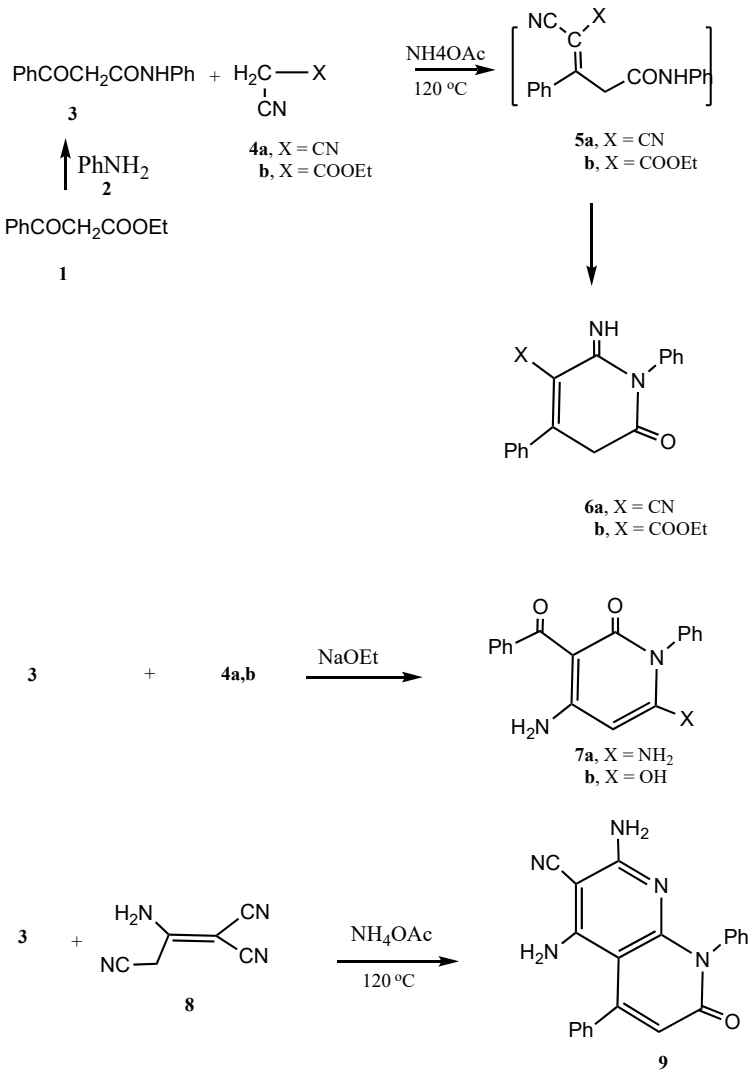
[a]NUGC, gastric cancer, DLDI, colon cancer, HA22T, liver cancer, HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; HR, gastric cancer; MCF, breast cancer; WI38, normal fibroblast cells.

Table 2. c-Met enzymatic activity of the most active compounds.

Compound No.	IC_{50} (nM) c-Met
6b	0.24 ± 0.05
7b	0.36 ± 0.28
9	0.42 ± 0.16
13a	0.58 ± 0.28
13b	0.61 ± 0.31
16a	0.48 ± 0.19
20b	0.53 ± 0.20
20c	0.86 ± 0.45
24b	0.50 ± 0.28
25	0.73 ± 0.25
26b	0.80 ± 0.32
	Foretinib1.16 ± 0.17

RESULTS AN DISCUSSION

The present investigation emphasized mainly on two important things, of these one is to the synthesis of molecules having nitrogen and or sulfur heterocyclic and the other is to determine

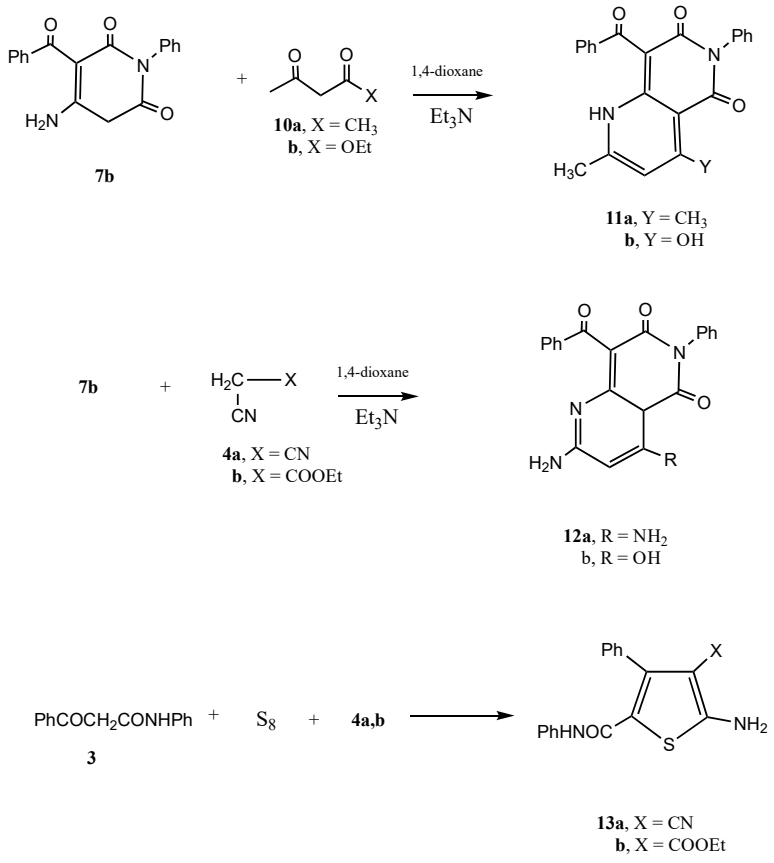


Scheme 1. Synthesis of compounds **6a**,**b**; **7a**,**b** and **9**.

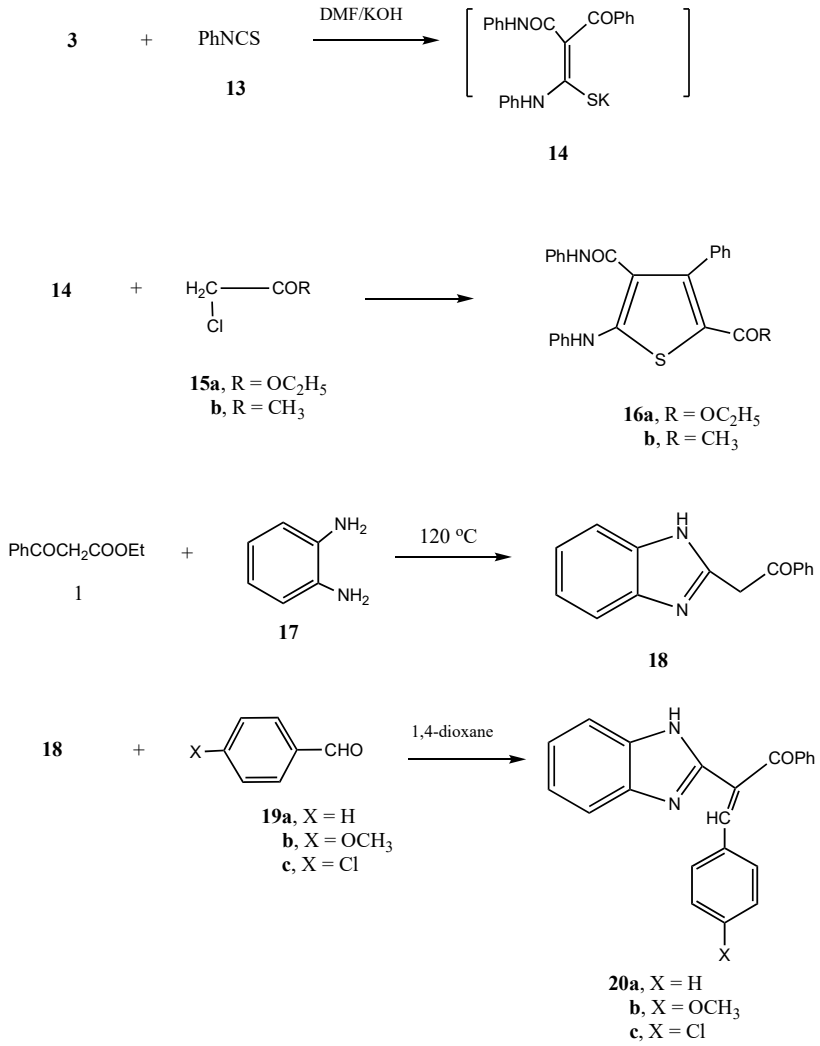
their cytotoxicity against cancer and normal cell lines. The synthetic strategies adopted for the synthesis of the intermediates and target compounds are depicted in Schemes 1-4. Nitrogen containing heterocyclic organic compounds having extra keto group show interesting chemical properties as well as biological activity [33]. The reaction of ethyl benzoylacetate (**1**) with aniline (**2**) gave the 3-oxo-N,3-diphenylpropanamide (**3**). Compound **3** reacted with either malononitrile (**4a**) or ethyl cyanoacetate (**4b**) to give the 1,2-dihydropyridinederivatives **6a** and **6b**, respectively, through the acyclic intermediates **5a,b**. The structure of the latter products was based on analytical and spectral data. Thus, the ^{1}H NMR spectrum of **6a** showed the presence of a singlet at δ 4.15 ppm indicating the presence of the CH_2 group, a multiplet at δ 7.30-7.99 ppm

for the two phenyl groups and a singlet at δ 10.21 ppm (D_2O exchangeable) corresponding to the NH group. Moreover, the ^{13}C NMR spectrum showed signal at δ 49.5 corresponding to the pyridine C-3, two signals at δ 103.6, 104.5 for the C=C group, a signal at δ 119.5 for the CN group, eight signals at δ 120.19, 132.9, 125.8, 129.4, 129.5, 134.5, 136,8, 139.5 equivalent to two benzene and pyridine C and two signals at δ 165.9, 195.2 confirming the C=O and C=N groups. On the other hand, the reaction of compound **3** with either malononitrile or ethyl cyanoacetate in sodium ethoxide solution gave the 1,6-dihydropyridine derivatives **7a** and **7b**, respectively. The reaction of compound **3** with the 2-aminoprop-1-ene-1,1,3-tricarbonitrile **8** gave the 7,8-dihydro-1,8-naphthyridine derivative **9** (Scheme 1) its structure was confirmed on the basis of analytical and spectral data.

The high yield of compound **7b** encouraged us to make further work in order to produce pharmaceutically active fused pyridine derivatives. Thus, the reaction of compound **7b** with either acetylacetone (**10a**) or ethyl acetoacetate (**10b**) gave the pyrido[2,3-*c*]pyridine derivatives **11a** and **11b**, respectively. Furthermore, compound **7b** reacted with either malononitrile or ethyl cyanoacetate to give the pyrido[2,3-*c*]pyridine derivatives **12a** and **12b**, respectively. The analytical and spectral data of **11a,b** and **12a,b** were in agreement with their proposed structures.



Scheme 2. Synthesis of compounds **11a**,**b**; **12a**,**b** and **13a,b**.

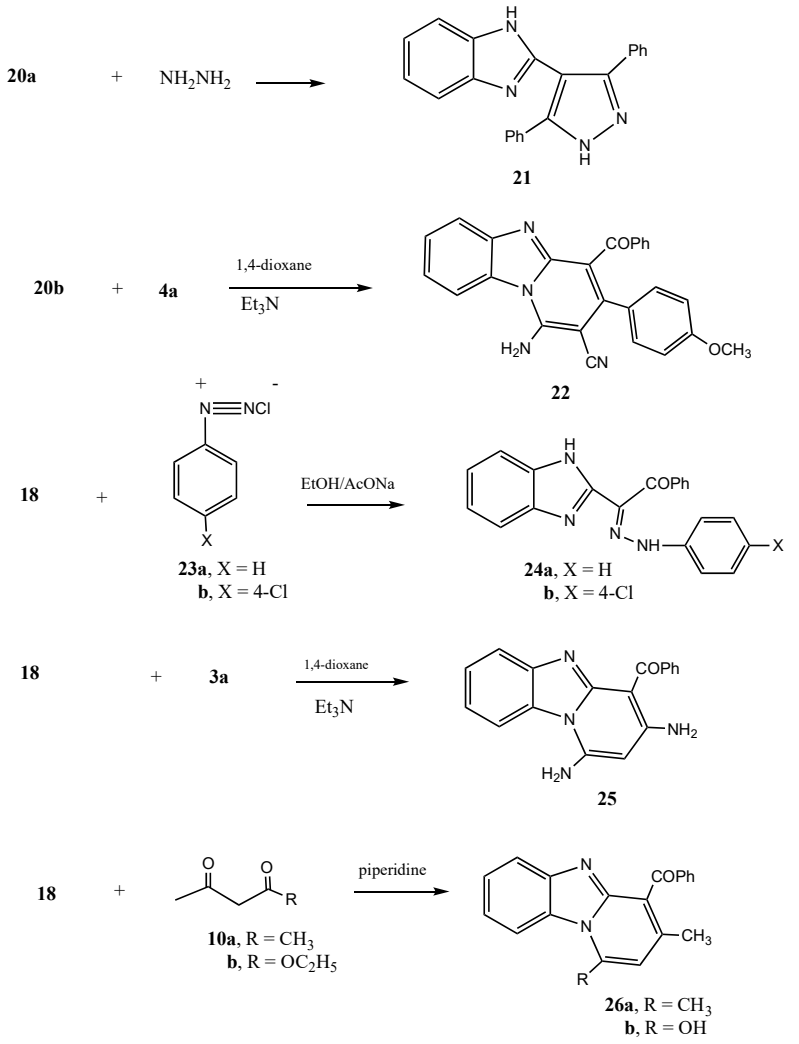


Scheme 3. Synthesis of compounds **16a**,**b**; **19** and **20a-c**.

The benzoylbenzanilide was studied to synthesis thiophene through the well known Gewald's thiophene synthesis [34, 35]. Thus, the reaction of compound **3** with elemental sulfur and either malononitrile (**4a**) and ethyl cyanoacetate (**4b**) gave the thiophene derivatives **13a** and **13b**, respectively (Scheme 2). On the other hand, compound **3** reacted with phenyl isothiocyanate in basic dimethylformamide gave the intermediate potassium sulphide salt **14**. The latter intermediate reacted with either ethyl chloroacetate (**15a**) or chloroacetone (**15b**) to give the thiophene derivatives**16a** and **16b**, respectively. The structures of the latter products were established on the basis of analytical and spectral data. Thus, the ^{1}H NMR spectrum of **16a** showed a triplet at δ 0.71 ppm indicating the presence of the ester CH_3 group, a quartet at δ 3.87 for the ester CH_2 group, a mutiplet at δ 7.43-7.49 equivalent to the three phenyl protons and two singlets at δ 8.20 and 10.39 for the two NH groups. The ^{13}C NMR spectrum showed the

presence of a signal at δ13.6 for the CH_3 group, a signal at δ 39.6 equivalent to the CH_2 group and eleven signals at δ110.0, 121.8, 125.8, 126.3, 128.4, 129.1, 130.4, 133.1, 138.0, 139.2, 140.2, 148.7 indicating the two C_6H_5 and thiophene C and two signals at δ165.8, 195.1 for the two C=O groups.

Next we moved towards the uses of ethyl benzoylacetate to synthesis of benzimidazole derivatives. Thus, compound **1** reacted with orthophenylene diamine in an oil bath 120 °C to give the 2-(1H-benzo[*d*]imidazol-2-yl)-1-phenylethanone (**18**). The analytical and spectral data of compound **18** were in agreement with its proposed structure. Compound **18** reacted with either benzaldehyde (**19a**), 4-methoxybenzaldehyde (**19b**) or 4-chlorobenzaldehyde (**19c**) to give the benzalidene derivatives **20a-c**, respectively (Scheme 3).



Scheme 4. Synthesis of compounds **21**; **24a**,**b**; **25** and **26a**,**b**.

Compound **20a** reacted with hydrazine hydrate to give the pyrazole derivative **21**. On the other hand, **20b** reacted with malononitrile (**3a**) to give the 1-amino-4-benzoyl-3-(4-methoxyphenyl)benzo[4,5]imidazo[1,2-*a*]pyridine-2-carbonitrile (**22**).

Compound **18** reacted with either benzenediazonium chloride (**23a**) or 4-chlorobenzenediazonium chloride (**23b**) to give the phenylhydrazo derivative **24a** and **24b**, respectively. On the other hand, it reacted with malononitrile (**3a**) in 1,4-dioxane containing triethylamine to give the benzo[4,5]imidazo[1,2-*a*]pyridine derivative **25**. Similarly compound **18** reacted with either either acetylacetone (**10a**) or ethyl acetoacetate (**10b**) to give the benzo[4,5]imidazo[1,2-*a*]pyridine derivatives **26a** and **26b**, respectively (Scheme 4).

Structure activity relationship

From Table 1 the newly synthesized compounds were tested against the six cancer cell lines the human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and a normal fibroblast cells (WI38). Compounds **6b**, **7b**, **9**, **13a**, **13b**, **16a**, **20b**, **20c**, **24b**, **25** and **26b** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. Comparing the cytotoxicity of the 1,2-dihydropyridine derivatives **6a** and **6b**, it is obvious that the ethyl ester group present in **6b** is responsible for its high cytotoxicity. On the other hand considering the 1,6-dihydropyridine **7a** and **7b**; the latter compound with the OH group showed high cytotoxicity against the NUGC, DLDI, HA22T and HONE1 cell lines. The 2-(amino(6-hydroxy-2-imino-1,4-diphenyl-1,2-dihydropyridin-3-yl)methylene)-malononitrile (**9**) showed high cytotoxicity against NUGC, DLDI and HEPG2 cell lines. Such high potency of compound **9** is attributed to the presence of the dicyanethylidene moiety. Comparing the cytotoxicity of the 1,6-naphthridine derivatives **11a** and **11b**, it is obvious that the presence of the OH group in compound **11b** is responsible for its high cytotoxicity against HA22T and HEPG2 cell lines. Such finding was confirmed through the comparison of the cytotoxicity of compounds **12a** and **12b**, where the last one showed higher cytotoxicity towards the six cancer cell lines. The significant cytotoxicity of **12b** appeared against the HEPG2 cell line with 870 nM.

For the thiophene derivatives **13a,b** and **16a,b**, it is clear from Table 1 that such compounds showed significant cytotoxicity against the cancer cell lines. Compounds **13b** and **16a** with the special optimal cytotoxicity which is attributed to the presence of the oxygen rich COOEt and OCH_3 moeties in both compound, respectively. It is of great interest to compare the cytotoxicity of the un-substituted benzimidazole derivative **18** and the benzylidene derivatives **20a-c**. It is obvious that compound **18** showed lower cytotoxicity relative to **20a-c**. In addition going through the latter compounds, one can notice that the presence of the electronegative groups OCH_3 and Cl in **20b** and **20c**, respectively are responsible for the high cytotoxicity of such compounds. Moreover, the Cl group showed more potency than the OCH_3 group as cleared from the greater cytotoxicity of **20c** over **20b**. The annulated compound **22** showed higher cytotoxicity than the pyrazole derivative **21**. The reaction of the benzimidazole derivative **18** with arylidenediazonium salts **23a** and **23b** gave the arylhydrazone derivatives **24a** and **24b** leading to a remarkable increase of cytotoxicity of **24a,b**. In addition compound **24b** with Cl group showed an optimal cytotoxicity against NUGC, DLDI, HA22T, HEPG2 and MCF cell lines with IC_{50}'s 380, 122, 33, 669 and 890 nM, respectively. It is of great value to note that compound **24b** with the Cl group showed more potency than **24a**. Considering the imidazo[1,2-*a*]pyridine derivatives **25** and **26a,b** compound **26b** showed the highest cytotoxticity among the three compounds. Moreover, the nitrogen rich compound **25** showed higher cytotoxicity than **26a**. It is of good worthy to notice that the ethyl 6-hydroxy-2-imino-1,4-diphenyl-1,2-dihydropyridine-3-carboxylate (**6b**) and the (1-hydroxy-3-methylbenzo[4,5]imidazo[1,2-*a*]pyridine-4-yl)(phenyl)methanone (**26b**) showed the maximum inhibitory effect towards the six cancer cell lines among the tested compounds.

It is very clear from our present finding that the heterocyclic systems with halogen substituted pattern OCH_3, Cl or COOEt show greater cytotoxic property. In every case it was observed that molecules with electronegative substitutions as compounds **6b**, **7b**, **13a,b**; **16a,b**; **20b**, **20c**, **25**, and **26b** showed higher cytotoxicity because they were bearing either oxygen or chlorine substituted as well as comprised with similar structural features.

Toxicity

It is well known that bioactive compounds are often toxic to shrimp larvae. Thus, in order to monitor these chemicals' *in vivo* lethality to shrimp larvae (*Artemia salina*), results are given in Table 3 for the compounds which exhibited optimal cytotoxic effect against cancer cell lines which are the ten compounds **6b**, **7b**, **13a**, **13b**, **16a**, **16b**, **20b**, **20c**, **25** and **26b**. The shrimp lethality assay is considered as a useful tool for preliminary assessment of toxicity, and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacteria toxins, pesticides, and cytotoxicity testing of dental materials, natural and synthetic organic compounds [36].

Table 3. Toxicity of the most cytotoxic compounds.

Compound No.	Cons. (µg/mL)	Mortality[a]	Toxicity	LC_{50}	Upper 95% limit	Lower 95% limit
6b	10 100 1000	0 0 8	Non toxic	890.22	-	-
7b	10 100 1000	2 6 8	Harmful	177.63	673.42	285.41
13a	10 100 1000	0 5 10	Harmful	213.29	66.12	22.45
13b	10 100 1000	1 4 10	Harmful	237.39	230.62	66.80
16a	10 100 1000	1 6 10	Harmful	122.32	240.31	107.82
16b	10 100 1000	0 1 10	Harmful	84.08	140.83	-
20b	10 100 1000	0 6 10	Harmful	238.40	166.23	215.80
20c	10 100 1000	4 8 10	Very toxic	12.40	-	-
25	10 100 1000	2 5 8	Very toxic	24.40	365.70	213.79
26b	10 100 1000	0 0 6	Non toxic	1000.2	-	-

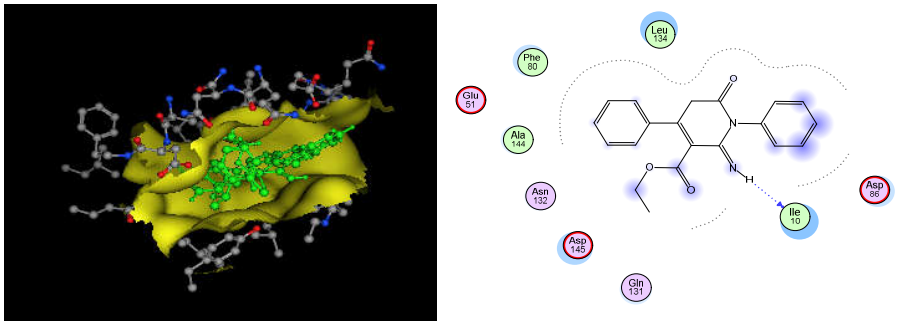
[a]Ten organisms (*A. salina*) tested for each concentration.

In order to prevent the toxicity results from possible false effects originated from solubility of compounds and DMSO's possible toxicity effect, compounds were prepared by dissolving in DMSO in the suggested DMSO volume ranges. It is clear from Table 3 that compounds **6b** and **26b** showed non toxicity against the tested organisms.

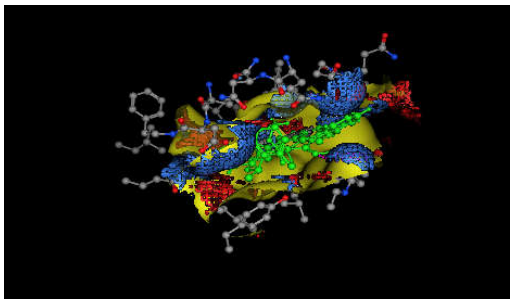
Molecular docking

The molecular studies were carried out using Molecular Operating Environment (MOE 2014). All the minimizations were performed with MOE until a RMSD gradient of 0.01 kcal/mol Å with MMFF94X force field and the partial charges were automatically calculated. Docking simulations were performed using the crystal structure of NUGC (PDB ID: 4ASD) which obtained from Protein Data Bank. Enzyme structure was checked for missing atoms, bonds and contacts. Water molecules was removed. Protonate 3D application of MOE was used to add the missing hydrogens and properly assign the ionization states. The ligand molecules were constructed using the builder molecule and were energy minimized. The active site was generated using the MOE-Alpha site finder. Ligands were docked within the active sites using the MOE-Dock The generated poses were energy minimized using the MMFF94x force field. Finally, the optimized poses were ranked using the GBVI/WSA DG free-energy estimates. Docking poses were visually inspected and interactions with binding pocket residues were analyzed.

Docking simulation was carried out to illustrate the binding mode and the interaction of the active compounds **6b**, **7b**, **13a** and **16b** with the amino acids in the active site of the NUGC. Docking study was performed using the crystal structure of NUGC (PDB ID: 4ASD) [20] which has co-crystallized ligand (sorafenib, BAX) as inhibitor inside its active site. In the beginning, docking study was validated by re-docking of co-crystallized ligand (BAX) inside the active site of NUGC. The re-docking of the co-crystallized ligand, BAX, was carried out to indicate the suitability of the used protocol for the planned docking study. The validation method was achieved by removing the bound ligand from the complex followed by its docking back into the binding site, which yielded root mean square deviation values RMSD of 0.88 Å with energy score (S) -9.94 kcal/mol. The top pose obtained from the MOE docking simulation showed the interactions of the co-crystallized ligand, BAX, with the key amino acids inside the active site. Docking ligand interaction and electrostatic map of compounds **6b, 7b, 13a** and **16b** were indicated through Figures 2-5, respectively.

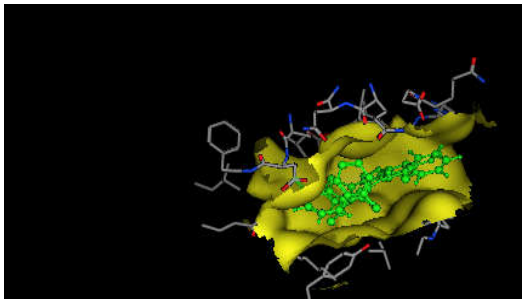


(A) (B)

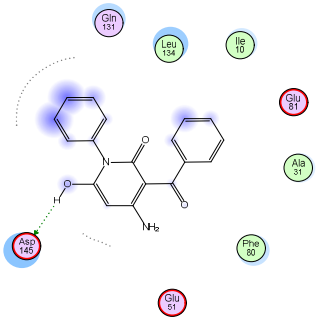


(C)

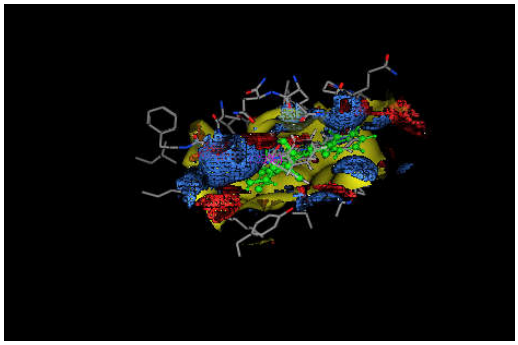
Figure 2. (A) Docking of compound **6b**, (B) Ligand interaction of compound **6b**, and (C) electrostatic map of compound **6b**. Energy of compound **6b** = -24.66.



(A)



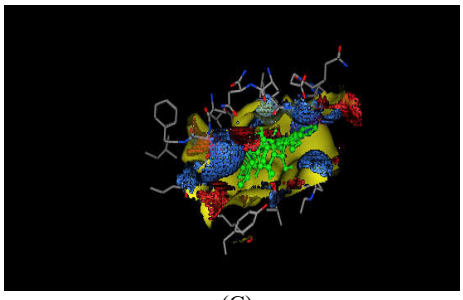
(B)



(C)

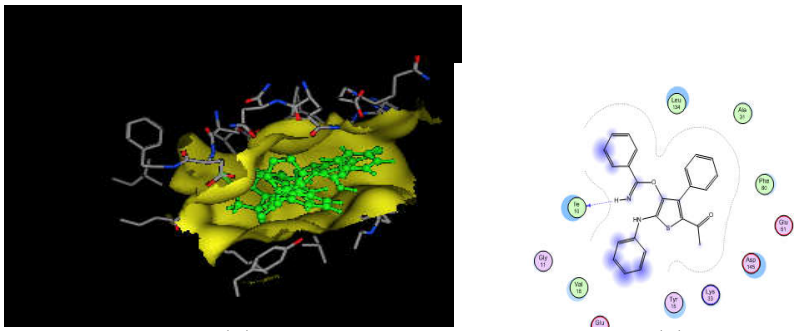
Figure 3. (A) Docking of compound **7b**, (B) ligand interaction of compound **7b**, and (C) electrostatic map of **7b**. Energy of compound **7b** = -20.26.

(A) (B)

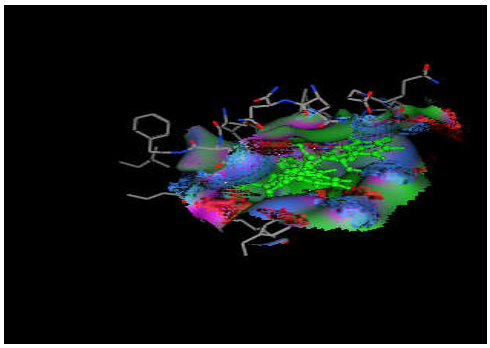


(C)

Figure 4. (A) Docking of compound **13a**, (B) ligand interaction of compound **13a**, and (C) electrostatic map of compound **13a**. Energy of compound **13b** = -24.89.



(A) (B)



(C)

Figure 5. (A) Energy of compound **16b**, (B) ligand interaction of compound **16b**, and (C) electrostatic map of compound **16b**. Energy of compound **16b** = -26.98.

For Figures 2-5 the blue color indicating the structure of the target molecule and the green color for the amino acid.

CONCLUSION

The present work, through simple synthetic approaches, led to the development of novel pyridine, thiophene and imidazole derivatives that exhibited remarkable antitumor activities against six tumor cell lines. As new class of heterocyclic compounds, eleven of the obtained new compounds showed remarkable cytotoxicity against most of the six tumor cell lines. Most of the active compounds were devoid of the typical nitrogen and or sulfur feature of heterocyclic derivatives and so the activity could be attributed to some sort of electronegative substituents through the synthesized compounds. In addition c-Met kinase inhibitions for the most active compounds showed that all compounds exhibited inhibitions higher than the reference drug foretinib. The results of the biological screening will encourage future work of heterocyclic compounds derived from β-diketones.

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