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

Rafat M. Mohareb1*, Mona M. Kamel2 and Yara R. Milad2,3

1Department of Chemistry, Faculty of Science, Cairo University, Giza, Egypt
2Department of Pharmaceutical Organic Chemistry, Faculty of Pharmacy, Cairo University, P.O. BOX 11562, Cairo, Egypt
3Jean Coutu, 531 Jarry Est, Montreal Quebec, Canada

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ABSTRACT. The reaction of the 3-oxo-N,N3-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-1,3-tricarbonitrile to give the pyridine derivative 9. Compound 7b reacted with the active methylene derivatives 10a,b and 4a,b to give the naphthydine 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 benzoylacetaet (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 forcinib as the reference drug where all compounds expressed higher activity than the reference drug.

KEY WORDS: Ethyl benzoylacetaet, 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 various 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 large number 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

*Corresponding author. E-mail: rafatmohareb@yahoo.com
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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 derivatives 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].

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 benzoyleacetate 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 $\nu_{max}$ 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$_6$H$_5$), 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. calcld. for C$_{18}$H$_{13}$N$_3$O (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 $\nu_{max}$ 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$_2$CH$_3$), 4.18 (q,
2H, J = 7.03 Hz, OCH$_2$CH$_3$), 5.95 (s, 2H, CH$_2$), 7.30-7.99 (m, 10H, 2C$_7$H$_5$), 10.21 (s, 1H, D$_2$O exchangeable, NH). $^1$C NMR (DMSO-d$_6$, 75 MHz) δ: 13.6 (OCH$_2$CH$_3$), 50.3 (OCH$_2$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. 1,6-Diazacyclohexane (2C=O), EIMS m/z 334 [M$^+$] (28). Anal. calcd. for C$_{25}$H$_{27}$N$_2$O$_3$ (334.38): C, 70.80; H, 4.88; N, 9.05. Found: C, 71.18; H, 4.87; N, 9.04.

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 6 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 $\nu_{max}$ 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), 6.15 (s, 1H, pyridine H-4), 7.31-7.68 (m, 10H, 2C$_7$H$_5$). $^{13}$C NMR (DMSO-d$_6$, 75 MHz): δ = 119.5, 120.1, 120.7, 122.0, 123.6, 124.7, 126.7, 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$_{19}$H$_{17}$N$_2$O (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 $\nu_{max}$ 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), 6.11 (s, 1H, pyridine H-4), 7.28-7.67 (m, 10H, 2C$_7$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$_{19}$H$_{17}$N$_2$O (306.52): 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 $\nu_{max}$ 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), 6.80 (s, 1H pyridine H-6), 7.31-7.58 (m, 10H, 2C$_7$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$_7$H$_5$, two pyridine C), 162.4 (C=O), 169.5 (C=N). EIMS m/z 353 [M$^+$] (22). Anal. calcd. for C$_{22}$H$_{17}$N$_2$O (353.39): C, 71.38; H, 4.25; N, 19.82. Found: C, 71.42; H, 4.31; N, 19.72.

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

Either acetylacetone (1.00 g, 0.01 mol) or ethyl acetocetate (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.

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8-Benzoyl-2,4-dimethyl-6-phenyl-1,6-naphththridine-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. ¹H NMR (DMSO-d₆, 400 MHz) δ = 2.51, 4.1 (2s, 6H, 2CH₃), 5.95 (s, 1H, pyridine H-3), 7.25-7.54 (m, 10H, 2C₆H₅), 10.16 (s, 1H, D₂O exchangeable, NH). ¹³C NMR (DMSO-d₆, 75 MHz) δ: 39.6, 49.8 (2 CH₃), 119.9, 120.0, 123.9, 124.2, 124.8, 125.8, 126.0, 126.3, 129.3, 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 C₂₁H₁₈N₂O₃ (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-naphththridine-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. ¹H NMR (DMSO-d₆, 400 MHz) δ = 2.51 (s, 3H, CH₃), 5.45 (s, 1H, pyridine H-3), 7.01-7.31 (m, 10H, 2C₆H₅), 8.20 (s, 1H, D₂O exchangeable, NH), 9.01 (s, 1H, D₂O exchangeable, OH). ¹³C NMR (DMSO-d₆, 75 MHz) δ: 25.1, (CH₃), 118.9, 119.8, 120.3, 123.6, 123.9, 124.2, 125.3, 125.6, 129.4, 131.2, 132.3, 134.2, 134.4 (two benzene, two pyridine C), 165.6, 172.0, 180.3 (3 C=O). EIMS m/z 372 [M⁺] (18). Anal. calcd. for C₂₁H₁₆N₂O₄ (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. ¹H NMR (DMSO-d₆, 400 MHz): δ = 3.58, 4.20 (2s, 4H, D₂O exchangeable, 2NH₃), 6.20 (s, 1H, pyridine H-3), 7.20 (s, 1H, pyridine H-3), 7.27-7.54 (m, 10H, 2C₆H₅). ¹³C NMR (DMSO-d₆, 75 MHz): δ: 48.9 (pyridine C-3), 119.9, 120.3, 120.6, 123.9, 125.8, 128.6, 129.3, 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 C₂₁H₁₆N₂O₃ (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. ¹H NMR (DMSO-d₆, 400 MHz): δ = 3.88 (s, 2H, D₂O exchangeable, NH₂), 4.14 (s, 1H, pyridine H-3), 7.27-7.67 (m, 11H, 2C₆H₅, pyridine H-3), 10.19 (s, 1H, D₂O exchangeable, OH). ¹³C NMR (DMSO-d₆, 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 C₂₁H₁₆N₂O₄ (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.

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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 νmax cm⁻¹: 3439-33259, 3056, 2923, 2211, 1687. ¹H NMR (DMSO-d₆, 400 MHz): δ = 4.15 (s, 2H, D₂O exchangeable, NH₂), 7.31-7.58 (m, 10H, 2C₂H₆), 10.21 (s, 1H, D₂O exchangeable, NH). ¹³C NMR (DMSO-d₆, 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 C₂₅H₁₇N₅OS (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 νmax cm⁻¹: 3426-3261, 3056, 2955, 2890, 1687-1661, 1604 (C=C). ¹H NMR (DMSO-d₆, 400 MHz) δ = 1.17 (t, 3H, J = 6.09 Hz, OCH₂CH₃), 4.18 (q, 2H, J = 6.09 Hz, OCH₂CH₃), 5.99 (s, 2H, D₂O exchangeable, NH₂), 7.31-7.99 (m, 10H, 2C₂H₆), 10.01 (s, 1H, D₂O exchangeable, NH). ¹³C NMR (DMSO-d₆, 75 MHz) δ: 16.5 (OCH₂CH₃), 50.2 (OCH₂CH₃), 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 C₆H₅, thiophene C), 165.8, 167.1 (2C=O). EIMS m/z 366 [M⁺] (28). Anal. calcd. for C₂₅H₁₁N₅O₅S (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-3-carboxylate (16a). Yellow crystals (1,4-dioxane), yield 86% (3.80 g), mp 160-163 °C. IR νmax cm⁻¹: 3397-3229, 3048, 2986, 2890, 1688-1668, 1624. ¹H NMR (DMSO-d₆, 400 MHz) δ = 1.21 (t, 3H, J = 7.21 Hz, OCH₂CH₃), 3.87(q, 2H, J = 7.21 Hz, OCH₂CH₃), 7.43-7.49 (m, 15H, 3C₆H₅), 8.20, 10.39 (2s, 2H, D₂O exchangeable, 2NH). ¹³C NMR (DMSO-d₆, 75 MHz) δ = 16.6 (OCH₂CH₃), 50.6 (OCH₂CH₃), 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 C₆H₅ and thiophene C), 165.8, 167.1 (2C=O). EIMS m/z 366 [M⁺] (28). Anal. calcd. for C₂₅H₁₉N₂O₅S (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-(phenylcarbamoyl)thiophene-3-carboxamide (16b). Orange crystals (1,4-dioxane), yield 79% (3.25 g), mp 175-177 °C. IR νmax cm⁻¹: 3453-3422, 3054, 2932, 1720-1668. ¹H NMR (DMSO-d₆, 400 MHz): δ = 3.56 (s, 3H, CH₃), 7.24-7.56 (m, 15H, 3C₆H₅), 10.51, 11.30 (2s, 2H, D₂O exchangeable, 2NH). ¹³C NMR (DMSO-d₆, 75 MHz) δ: 16.6 (CH₃), 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 C₂₅H₁₉N₂O₅S (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-Benz[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-d₆, 400 MHz): δ = 3.50 (s, 2H, CH₃), 7.20-7.55 (m, 9H, C₆H₅, C₅H₄), 10.45 (s, 1H, D₂O exchangeable, NH). ¹³C NMR (DMSO-d₆, 75 MHz): δ = 49.8 (CH₃), 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 C₃₀H₂₅N₄ (326.27): 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-methoxybenzaldehyde (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-Benz[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-d₆, 400 MHz): δ = 6.06 (s, 1H, CH), 7.26-7.55 (m, 14H, 2C₆H₅, C₅H₄), 10.54 (s, 1H, D₂O exchangeable NH). ¹³C NMR (DMSO-d₆, 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 (2C₆H₅, C₅H₄), 160.2 (CO), 173.4 (C=N). EIMS m/z 324 [M⁺] (48); anal. calcd. for C₃₂H₂₆N₂O (324.38): C, 81.46; H, 4.97; N, 8.64. Found: C, 81.66; H, 5.26; N, 8.47.

2-(1H-Benz[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-d₆, 400 MHz) δ = 3.50 (s, 3H, CH₃), 6.08 (s, 1H, CH), 7.26-7.55 (m, 13H, C₆H₅, 2C₅H₄), 10.54 (s, 1H, D₂O exchangeable NH). ¹³C NMR (DMSO-d₆, 75 MHz) δ: 38.7 (CH₃), 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 (C₆H₅, 2C₅H₄), 161.2 (CO), 172.8 (C=N). EIMS m/z 354 [M⁺] (25). Anal. calcd. for C₃₃H₂₈N₂O₃ (354.41): C, 77.95; H, 5.12; N, 7.90. Found: C, 78.28; H, 5.08; N, 8.21.

2-(1H-Benz[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-d₆, 400 MHz) δ = 6.08 (s, 1H, CH), 7.26-7.55 (m, 13H, C₆H₅, 2C₅H₄), 10.54 (s, 1H, D₂O exchangeable NH). ¹³C NMR (DMSO-d₆, 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, 126.7, 128.6 (C₆H₅, 2C₅H₄), 161.4 (CO), 171.3 (C=N). EIMS m/z 358 [M⁺] (38); anal. calcd. for C₃₃H₂₈ClN₂O (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: 3340-3302, 3054, 1662, 1608. ¹H NMR (DMSO-d₆, 400 MHz) δ = 7.28-7.74 (m, 14H, 2C₆H₅, C₅H₄), 10.12, 11.20 (2s, 2H, D₂O exchangeable, 2NH). ¹³C NMR (DMSO-d₆, 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 (2C₆H₅, C₅H₄ pyrazole C), 163.4, 170.0 (two C=N). EIMS m/z 336 [M⁺] (66); anal. calcd. for C₃₅H₂₄N₄O (536.40): C, 78.55; H, 4.79; N, 16.66. Found: C, 78.39; H, 4.86; N, 16.41.

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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. ¹H NMR (DMSO-d₆, 400 MHz) δ = 3.50 (s, 3H, OCH₃), 5.02 (s, 2H, D₆O exchangeable, NH₂), 7.21-7.55 (m, 13H, C₆H₅, 2C₆H₅). ¹³C NMR (DMSO-d₆, 75 MHz) δ: 46.8 (OCH₃), 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 (C₆H₅), 163.2 (C=O), 166.8, 168.6 (C=N). EIMS m/z 340 [M⁺] (48); anal. calcd. for C₁₂H₁₂N₂O₂: C, 74.33; 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, 0.05 mol) either benzenediazonium chloride (0.01 mol) or 4-chlorobenzenediazonium 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-Benz[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: 3440-3292, 3053, 1668, 1610. ¹H NMR (DMSO-d₆, 400 MHz) δ = 7.26-7.55 (m, 14H, 2C₆H₅, 2C₆H₅), 8.05, 10.55 (2s, 2H, D₆O exchangeable, 2NH), ¹³C NMR (DMSO-d₆, 75 MHz) δ: 121.0, 121.4, 122.6, 123.1, 123.5, 123.9, 124.6, 125.0, 125.3, 126.0, 128.2, 128.4, 130.4, 130.9, 137.7, 139.9 (2C₆H₅, C₆H₅), 163.0 (C=O), 166.8, 168.4 (2C=N). EIMS m/z 340 [M⁺] (28); anal. calcd. for C₁₂H₁₀N₄O(340.13): C, 74.10; H, 4.74; N, 16.46. Found: C, 74.33; H, 4.59; N, 16.79.

2-(1H-Benz[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. ¹H NMR (DMSO-d₆, 400 MHz) δ = 7.26-7.58 (m, 13H, C₆H₅, 2C₆H₅), 8.08, 10.55 (2s, 2H, D₆O exchangeable, 2NH). ¹³C NMR (DMSO-d₆, 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 (C₆H₅), 163.2 (C=O), 166.8, 168.6 (2C=N). EIMS m/z 374 [M⁺] (56). Anal. calcd. for C₁₃H₁₀ClN₄O (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)methane (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. ¹H NMR (DMSO-d₆, 400 MHz) δ = 4.10, 5.40 (2s, 4H, D₆O exchangeable, 2NH), 7.26-7.55 (m, 10H, C₆H₅, C₆H₅, pyridine H-3). ¹³C NMR (DMSO-d₆, 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 (C₆H₅, pyridine C), 165.7 (C=O), 171.3 (C=N). EIMS: m/z = 302 [M⁺] (24); anal. calcd. for C₁₈H₁₄N₄O (302.34): C, 71.51; H, 4.67; N, 18.53. Found: C, 71.39; H, 4.48; N, 18.71.

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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 νmax cm⁻¹: 3058, 1720, 1620. ¹H NMR (DMSO-d₆, 400 MHz) δ = 2.42, 3.34 (2s, 6H, 2CH₃), 6.61 (s, 1H, pyridine H-3), 7.27-7.36 (m, 9H, C₆H₅), 125.3, 125.8, 126.1, 127.3, 129.2, 130.4, 140.5, 143.0, 150.3 (C₆H₄, pyridine C), 165.5 (C=O), 167.2 (C=N). EIMS m/z 300 [M⁺] (68). Anal. calcd. for C₂₁H₁₆N₄O (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 νmax cm⁻¹: 3435-3125, 3058, 1696, 1474. ¹H NMR (DMSO-d₆, 400 MHz) δ = 3.34 (s, 3H, CH₃), 6.63 (s, 1H, pyridine H-3), 7.27-7.38 (m, 9H, C₆H₅, C₆H₄), 10.50 (s, 1H, D₂O exchangeable, OH). ¹³C NMR (DMSO-d₆, 75 MHz) δ = 24.1 (CH₃), 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 (C₆H₄, C₆H₅, pyridine), 166.4 (C=O), 168.2 (C=N). EIMS m/z 302 [M⁺] (28); anal. calcd. for C₁₉H₁₄N₂O₂ (302.33): C, 75.48; H, 4.67; N, 9.27. Found: C, 75.21; H, 4.59; N, 9.41.

Chemicals

Chemicals 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 IC₅₀’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 IC₅₀’s much higher than that of the reference foretinib.
Uses of β-diketones for the synthesis of novel heterocyclic compounds

Table 1. Cytotoxicity of the newly synthesized compounds against a variety of cancer cell lines [IC50 (nM)].

| Compound | 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  | 307b  | 670   | 2742| na   |
| 26b      | 32   | 48   | 250   | 82    | 71    | 559 | na   |
| CHS 828  | 25   | 2315 | 2067  | 1245  | 15    | 18  | na   |

*NUGC, gastric cancer, DLDI, colon cancer, HA22T, liver cancer, HEPG2, liver cancer; HONE1, 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. | IC50 (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|       |
| Foretinib    | 1.16 ± 0.17|       |

RESULTS AND 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

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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 benzoyleacetate (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 $\delta$ 4.15 ppm indicating the presence of the CH$_2$ group, a multiplet at $\delta$ 7.30-7.99 ppm
for the two phenyl groups and a singlet at δ 10.21 ppm (D$_2$O 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, 123.9, 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 benzyolbenzanilide 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 $\delta$ 0.71 ppm indicating the presence of the ester CH$_3$ group, a quartet at $\delta$ 3.87 for the ester CH$_2$ group, a multiplet at $\delta$ 7.43-7.49 equivalent to the three phenyl protons and two singlets at $\delta$ 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 CH₃ group, a signal at δ 39.6 equivalent to the CH₂ 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 C₆H₅ 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 benzoylacetae 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.

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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-α]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-α]pyridine derivative 25. Similarly compound 18 reacted with either ethyl acetoacetate (10a) or ethyl acetooacetate (10b) to give the benzo[4,5]imidazo[1,2-α]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 (W138). Compounds 6b, 7b, 9, 13a, 13b, 16a, 20b, 20c, 24b, 25 and 26b exhibited optimal cytotoxic effect against cancer cell lines, with IC₅₀’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, DLD1, 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, DLD1 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 OCH₃ 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 OCH₃ 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 OCH₃ 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 arylhydrazine 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, DLD1, HA22T, HEPG2 and MCF cell lines with IC₅₀’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. Comparing the imidazo[1,2-α]pyridine derivatives 25 and 26a,b compound 26b showed the highest cytotoxicity 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-α]pyridine-4-yl)(phenyl)methanone (26b) showed the maximum inhibitory effect towards the six cancer cell lines among the tested compounds.

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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.
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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.

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.
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.
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**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.

**REFERENCES**

1. Crooks, P.A.; DwoskinmL. P. Contribution of CNS nicotine metabolites to the neuropharmacological effects of nicotine and tobacco smoking. *Biochem. Pharm.* 1997, 54, 743-753.
2. Jatav, V.; Mishra, P.; Kashaw, S.; Stables, J. P. CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3H)-ones. *Eur. J. Med. Chem.* 2008, 43, 1945-1954.
3. Warren, B.K.; Knaus, E.E. Pyridine and reduced pyridine analogues of 10H-pyrido[3,4-b][1,4]benzothiazines with analgesic activity. *Eur. J. Med. Chem.* 1987, 22, 411-415.
4. Mulchin, B.J.; Newton, C.G.; Baty, J.W.; Grasso, C.H.; Martin, W.J.; Walton, M.C.; Dangerfield, E.M.; Plunkett, C.H.; Berridge, M.V.; Harper, J.L.; Timmer, M.S.M.; Stocker, B.L. The anti-cancer, anti-inflammatory and tuberculostatic activities of a series of 6,7-substituted-5,8-quinolinequinones. *Bioorg. Med. Chem.* 2010, 18, 3238-3251.
5. Sangshetti, J.N.; Dharmadhikari, P.P.; Chouthe, R.S.; Fatema, B.; Lad, V.; Karande, V.; Sunil, N.; Darandale, S.N.; Devanand, B.; Shinde, D.B. Microwave assisted nano (ZnO–TiO2) catalyzed synthesis of some new 4,5,6,7-tetrahydro-6-((5-substituted-1,3,4-oxadiazol-2-yl)methyl)thieno[2,3-c]pyridine as antimicrobial agents. *Bioorg. Med. Chem. Lett.* 2013, 23, 2250-2253.

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6. Witherington, J.; Bordas, V.; Garland, S.L.; Hickey, D.M.; Ife, R.J.; Liddle, J.; Saunders, M.; Smith, D.G.; Ward, R.W. 5-Aryl-pyrazolo[3,4-b]pyridines: potent inhibitors of glycogen synthase kinase-3 (GSK-3). *Bioorg. Med. Chem. Lett.* 2003, 13, 1577-1580.

7. Liu, X.H.; Liu, H.F.; Shen, X.; Song, B.A.; Bhadury, P.S.; Zhu, H.L.; Liu, J.X.; Qi, X.B. Synthesis and molecular docking studies of novel 2-chloro-pyridine derivatives containing flavone moieties as potential antitumor agents. *Bioorg. Med. Chem. Lett.* 2010, 20, 4163-4167.

8. Ram, S. New benzimidazole carbamates as antifilarial agents. *Prog. Med. Chem.* 1988, 25, 233-247.

9. Gaba, M.; Singh, S.; Mohan, C. Benzimidazole: an emerging scaffold for analgesic and anti-inflammatory agents. *Eur. J. Med. Chem.* 2014, 76, 494-505.

10. Nevado, J.J.B.; Peñalvo, G.C.; Dorado, R.M.R.; Robledo, V.R. Simultaneous determination of omeprazole and their main metabolites in human urine samples by capillary electrophoresis using electrospray ionization-mass spectrometry detection. *J. Pharm. Biomed. Anal.* 2014, 92, 211-219.

11. Natsuki Ishizuka, N.; Ken-ichi Matsumura, K.I.; Katsunori Sakai, K.; Masafumi Fujimoto, M.; Shin-ichi Mihara, S.I.; Teruo Yamamori, T. Structure–activity relationships of a novel class of endothelin-A receptor antagonists and discovery of potent and selective receptor antagonist, 2-(benzo[1,3]dioxol-5-yl)-6-isopropoxy-4-(4-methoxyphenyl)-2H-chromene-3-carboxylic acid (S-1255). 1. Study on structure–activity relationships and basic structure crucial for ETA antagonism. *J. Med. Chem.* 2002, 45, 2041-2055.

12. Khoje, A.D.; Charnock, C.; Wan, B.; Franzblau, S.; Gundersen, L.L. Synthesis and antimycobacterial activities of non-purine analogs of 6-aryl-9-benzylpurines: Imidazopyridines, pyrrolopyridines, benzimidazoles, and indoles. *Bioorg. Med. Chem.* 2011, 19, 3483-3491.

13. Seenaiah, D.; Reddy, P.R.; Reddy, G.M.; Padmaja, A.; Padmavathi, V.; Krishna, N.S. Synthesis, antimicrobial and cytotoxic activities of pyrimidinyl benzoazolone, benzothiazole and benzimidazole. *Eur. J. Med. Chem.* 2014, 77, 1-7.

14. Yadav, S.; Kumar, P.; Clercq, E.D.; Balzarini, J.; Pannecoque, C.; Dewan, S.K.; Narasimhan, B. 4-[1-(Substituted aryl/alkyl carbonyl)-benzimidazol-2-yl]-benzenesulfonic acids: Synthesis, antimicrobial activity, QSAR studies, and antiviral evaluation. *Eur. J. Med. Chem.* 2010, 45, 5985-5997.

15. Zhang, H.Z.; Damu, G.L.V.; Cai, G.X.; Zhou, C.H. Design, synthesis and antimicrobial evaluation of novel benzimidazole type of fluconazole analogues and their synergistic effects with chloromycin, norfloxacin and fluconazole. *Eur. J. Med. Chem.* 2013, 64, 329-344.

16. Dahiya, R.; Pathak, D. Synthetic studies on novel benzimidazoleopeptides with antimicrobial, cytotoxic and anthelmintic potential. *Eur. J. Med. Chem.* 2007, 42, 772-798.

17. Fonseca, T.; Gigante, B.; Marques, M.M.; Gilchrist, T.L.; Clercq, E.D. Synthesis and antiviral evaluation of benzimidazoles, quinoxalines and indoles from dehydroabietic acid. *Bioorg. Med. Chem.* 2004, 12, 103-112.

18. Herrmann, E.C.; Judith, A.; Herrmann, J.A.; Donald, C.; Delong, D.C. Comparison of the antiviral effects of substituted benzimidazoles and guanidine in vitro and in vivo. *Antiviral Res.* 1981, 1, 301-314.

19. Sharma, S.; Gangal, S.; Rauf, A. Convenient one-pot synthesis of novel 2-substituted benzimidazoles, tetrahydrobenzimidazoles and imidazoles and evaluation of their in vitro antibacterial and antifungal activities. *Eur. J. Med. Chem.* 2009, 44, 1751-1757.

20. Shingalapur, R.V.; Hosamani, K.M.; Keri, R.S. Synthesis and evaluation of in vitro antimicrobial and anti-tubercular activity of 2-styryl benzimidazoles. *Eur. J. Med. Chem.* 2009, 44, 4244-4248.
21. Pawar, N.S.; Dalal, D.S.; Shimpi, S.R.; Mahulikar, P.P. Studies of antimicrobial activity of N-alkyl and N-acyl 2-(4-thiazolyl)-1H-benzimidazoles. Eur. J. Pharm. Sci. 2004, 21, 115-118.
22. Rashid, M.; Husain, A.; Mishra, R. Synthesis of benzimidazoles bearing oxadiazole nucleus as anticancer agents. Eur. J. Med. Chem. 2012, 54, 855-866.
23. Shaharyar, M.; Abdullah, M.M.; Bakht, M.A.; Majeed, J. Pyrazoline bearing benzimidazoles: Search for anticancer agent. Eur. J. Med. Chem. 2010, 45, 114-119.
24. Abonia, R.; Cortés, E.; Insuasty, B.; Quiroga, J.; Nogueras, M.; Cobo, J. Synthesis of novel 1,2,5-trisubstituted benzimidazoles as potential antitumor agents. Eur. J. Med. Chem. 2011, 46, 4062-4070.
25. Rashid, M.; Husain, A.; Mishra, R.; Anjum, S. Eradication of toxic material by using green chemistry in the development of anticancer drugs. Toxicol. Lett. 2012, 63, 53-56.
26. Badawy, E.A.M.; Kappe, T. Benzimidazole condensed ring systems. XI. Synthesis of some substituted cycloalkyl pyrido[1,2-a]benzimidazoles with anticipated antineoplastic activity. Eur. J. Med. Chem. 1999, 34, 663-667.
27. Huang, S.T.; Hsei, I.J.; Chen, C. Synthesis and anticancer evaluation of bis(benzimidazoles), bis(benzoazoles), and benzothiazoles. Bioorg. Med. Chem. 2006, 14, 6106-6119.
28. Omar, M.A.; Shaker, Y.M.; Galal, S.A.; Ali, M.M.; Kerwin, S.M.; Li, J.; Tokuda, H. Ramadan, R.A.; El-Diwani, H.I. Synthesis and docking studies of novel antitumor benzimidazoles. Bioorg. Med. Chem. 2012, 20, 6989-7001.
29. Harapanhalli, R.S.; Howel, R.W.; Rao, D.V. Bis-benzimidazole dyes, Hoechst 33258 and Hoechst 33342: Radiolodination, facile purification and subcellular distribution. Nucl. Med. Biol. 1994, 21, 641-647.
30. Singh, S.; Dwarakanath, B.S.; Lazar, T.; Mathew, T.L. DNA ligand Hoechst-33342 enhances UV induced cytotoxicity in human glima cell lines. J. Photochem. Photobio. B: Biol. 2004, 77, 45-54.
31. Heba, S.A.; Elzahabi, H.A.S. Synthesis, characterization of some benzazoles bearing pyridine moiety: Search for novel anticancer agents. Eur. J. Med. Chem. 2011, 46, 4025-4035.
32. Tseng, J.R.; Stuart, D.; Aardalen, K.; Kaplan, A.; Aziz, N.; Hughes, N.P.; Gambhir, S.S. Use of DNA microarray and small animal positron emission tomography in preclinical drug evaluation of RAF265, a novel B-Raf/VEGFR-2 inhibitor. Neoplasia 2011, 13, 266-275.
33. Islam, M.R.; Mohsin, M. Synthesis of isatin, 5-chloroisatin and their Δ2-1,3,4 oxadiazoline derivatives for comparative cytotoxicity study on brine shrimp. Bangladesh. J. Pharmacol. 2007, 2, 7-12.
34. Bryan, P.; McKibben, B.P.; Craig, H.; Cartwright, C.H.; Arlindo, L.; Castelhano, A.L. Practical synthesis of tetrasubstituted thiophenes for use in compound libraries. Tetrahedron Lett. 1999, 40, 5471-5474.
35. Balamurugan, K.; Perumal, S.; Reddy, A.S.K.; Yogeeswari, P.; Sriram, D. A facile domino protocol for the regioelective synthesis and discovery of novel 2-amino-5-arylthieno[2, 3-b] thiophenes as antymyobacterial agents. Tetrahedron Lett. 2009, 50, 6191-6195.
36. Calleja, M.C.; Persoon, G. Cyst-based toxicity tests. IV. The potential of ecotoxicological tests for the prediction of acute toxicity in man as evaluated on the first ten chemicals of the MEIC programme. ATLA 1992, 20, 396-405.