Full Paper

Efficient Regioselective Synthesis and Potential Antitumor Evaluation of Isoxazolo[5,4-*b*]pyridines and Related Annulated Compounds

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The reaction of 5-amino-3-methylisoxazole with appropriate α,β-unsaturated ketones gave the corresponding isoxazolo[5,4-*b*]pyridines. Treatment of **1** with 2,6-dibenzylidenecyclohexanone or 2-benzylidenedimedone afforded the corresponding isoxazolo[5,4-*b*]quinoline derivatives. 4,6,8,9-Tetrahydroisoxazolo[5,4-*b*]quinolin-5-one derivative was also obtained by multicomponent condensation reaction of **1** with dimedone and benzaldehyde. Heterocyclic annulation of the isoxazolo[5,4-*b*]pyridine system was achieved via reaction of **1** with benzylidene derivatives of indandione, quinuclidone, pyrazolone, and oxazolone. A representative of some newly synthesized compounds was evaluated as antitumor agents.

Keywords: Annulation / Antitumor activity (*in vitro*) / Biselectrophiles / Isoxazole / Regioselective

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Introduction

The isoxazole rings are frequently present in biologically active compounds and are used as building blocks in the synthesis of new potential drugs. Therefore, 5-amino-3-methylisoxazole (**1**) acts as a building block for construction of isoxazole-annulated heterocyclic systems. Compound **1** has multiple competing sites for ring-annulation reaction toward the biselectrophiles. The 4-position of the isoxazole is the most nucleophilic and attacks the most electrophilic carbon atom of the reactants.

Cyclocondensation of aminoazoles and aminoazines with α,β-unsaturated ketones or their synthetic precursor aldehydes and ketones containing at least two active hydrogen atoms are the most widespread and investigated pathways to fused dihydroazaheterocycles [1, 2]. In most cases both types of reaction pathways, that is, a sequential protocol involving the initial synthesis of the α,β-unsaturated ketonic compounds and the other pathway is the three-component reaction, yield the same products [3]. However, in rare cases the direct multicomponent procedure may lead to the formation of different products [4], connected to the complex reaction mechanism of these transformations [5, 6]. It was felt interesting to synthesize fused heterocyclic systems incorporating isoxazole moiety and evaluate their biological activity. Our interest directed to synthesize pyridine carboxylic acid fused to isoxazole rings is mainly caused by the known biological activity of these systems [7].

Indandiones are very important synthons for synthesis of fused compounds containing the indandione moiety, which are evaluated as inhibitors of human papillomavirus type II [8, 9] and as antimicrobial agents [10].

Results and discussion

Chemistry

5-Amino-3-methylisoxazole (**1**) was reacted with (*E*)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (**2**) in DMF via cyclocondensation reaction to furnish the isoxazolo[5,4-*b*]pyridine **3**. We have found that condensation between **1** and (*E*)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (**2**) was regioselective and afforded **3** (TLC monitoring) which is concordant with similar reactions of aminoisoxazole and biselectrophiles [11] (Scheme 1).

The plausible reaction mechanism for the formation of **3** is displayed in Scheme 2.

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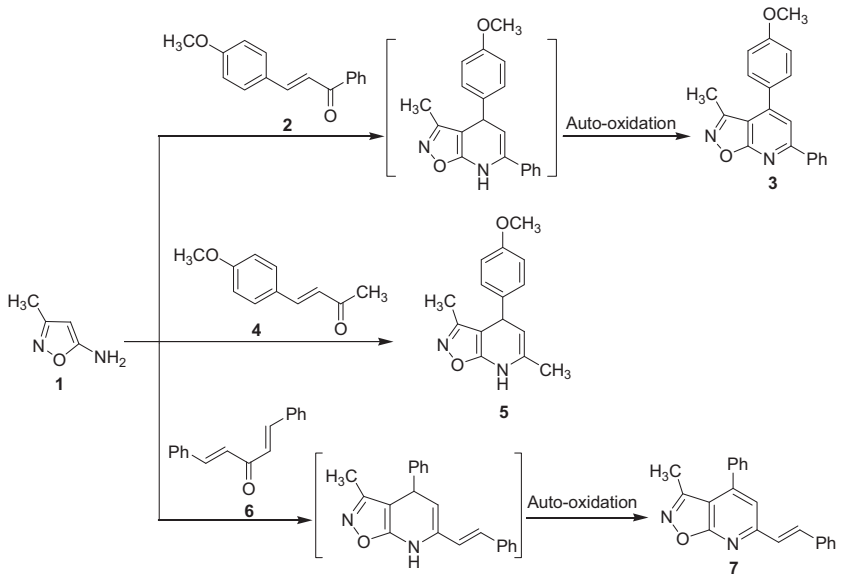
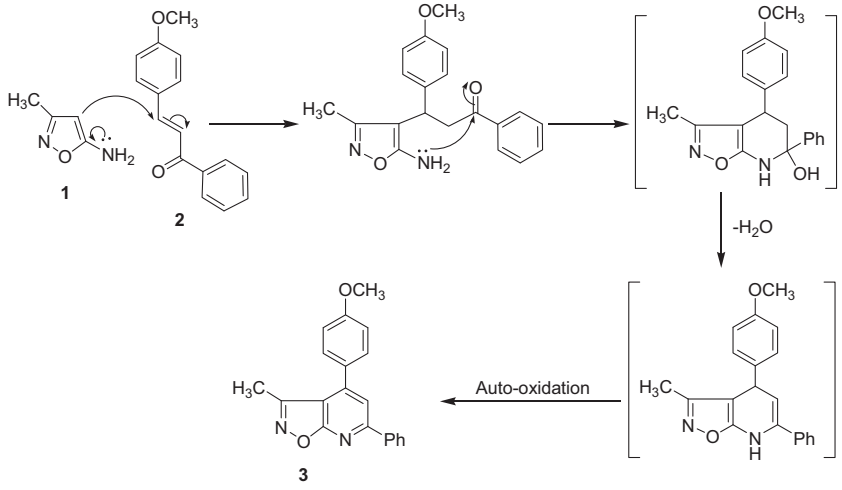


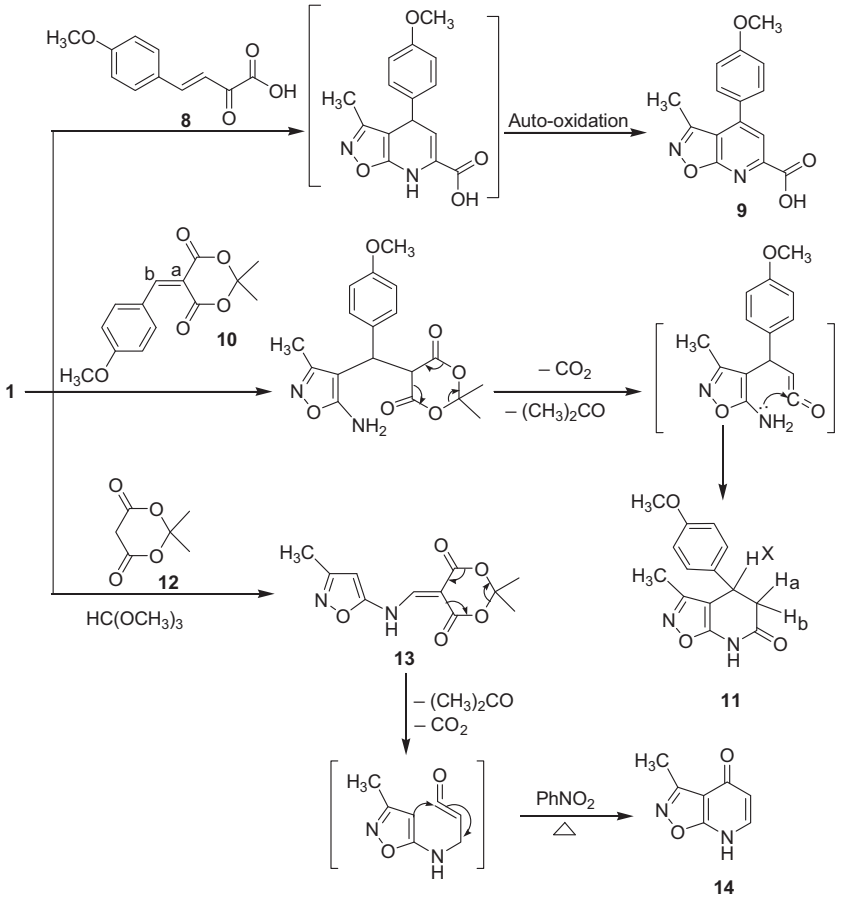
Scheme 1. Cyclocondensation reactions of **1** with α,β-unsaturated ketones.

In a similar manner, 4-(4-methoxyphenyl)-3,6-dimethyl-4,7-dihydro-isoxazolo[5,4-*b*]pyridine (**5**) was synthesized by the reaction of **1** with (*E*)-4-(4-methoxyphenyl)but-3-en-2-one (**4**) in DMF. An efficient method for selective synthesis of 6-styrylisoxazolo[5,4-*b*]pyridine derivatives **7** was achieved by the Micheal addition of **1** to 1,5-diphenyl-1,4-pentadiene-3-one (**6**) in DMF via cyclocondensation reaction (Scheme 1). The dihydropyridine adduct from Hantzsh synthesis afforded the fully oxidized (aromatized) pyridyl product presumably due to the highly conjugated system formed. The constitution of **7** was affirmed through its spectral data.

Treatment of (*E*)-4-(4-methoxyphenyl)-2-oxobut-3-enoic acid (**8**) with **1** in acetic acid led to 4-(4-methoxyphenyl)-3-methyl-isoxazolo[5,4-*b*]pyridine-6-carboxylic acid (**9**, Scheme 3). Moreover, we have provided an efficient method for the synthesis of fused heterocyclic compound containing the dihydroisoxazolo[5,4-*b*]pyridin-6-one skeleton **11**. Therefore, refluxing equimolar amounts of **1** and anisylidene Meldrum's acid (**10**) [12, 13] for 30 min afforded **11** in modest yield. In principle, the amine **1** attack on β-C of the α,β-unsaturated cyclic ester **10** was followed by cyclization reaction of the intermediate adduct to form **11**. The formation of **11** was in line with that reported in the literature to synthesize pyrazolo[5,4-*b*]pyridin-6-one [14], while an alternative route to synthesize **11** was reported by Tu et al. [15]. We assumed that formation of **11** was directed via initial addition of the β-carbon of the enamino group in compound **1** to the β-C of the cyclic ester and then loss of one molecule of



Scheme 2. The plausible mechanism for the formation of isoxazolo[5,4-*b*]pyridine derivative **3**.



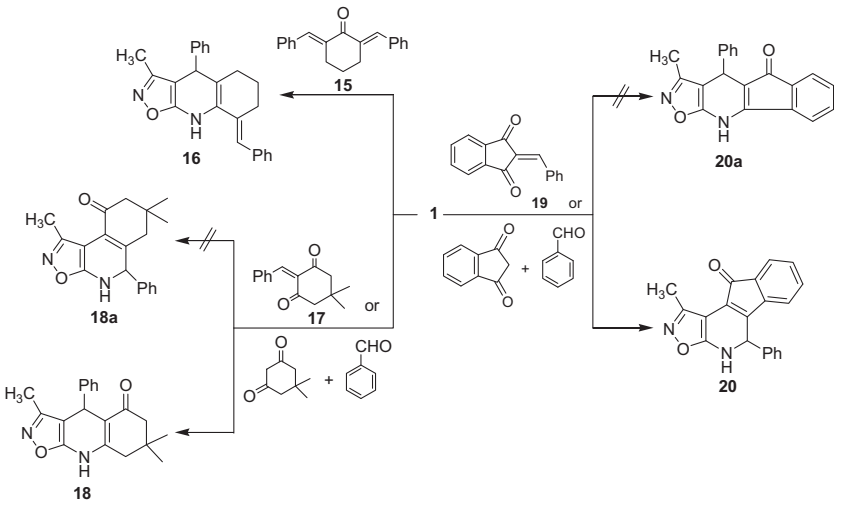
Scheme 3. Pyridine ring formation through auto-oxidation, nucleophile attack, and cyclization reactions.

acetone and CO_2 which leads to the formation of ketene intermediate followed by its cyclization. This behavior is well known for the thermolysis of Meldrum's acid derivatives [16, 17], and it is shown in Scheme 3. Spectroscopic data for **11** showed the expected distinctive features. Furthermore, following the procedure reported by Tu et al. [15] for 5-amino-3-aryl pyrazoles, refluxing a solution of Meldrum's acid **12** [18] and methyl orthoformate (1:5) followed by immediate addition of **1** in an equimolecular amount relative to Meldrum's acid furnished the corresponding 2,2-dimethyl-5-[(3-methyl-isoxazol-5-ylamino)methylene]-1,3-dioxane-4,6-dione (**13**). Its reflux in nitrobenzene afforded the dihydroisoxazolopyridine compound **14**.

In addition, the reaction of **1** with 2,6-dibenzylidenecyclohexanone (**15**) in DMF afforded 8-benzylidene-4,5,6,7,8,9-hexahydro-3-methyl-4-phenyl isoxazolo [5,4-*b*]quinoline (**16**). Dimedone derivative **17** is an attractive synthetic intermediate to develop an efficient and versatile synthesis of linear 3,7,7-trimethyl-4-phenyl-4,6,8,9-tetrahydroisoxazolo-[5,4-*b*]quinolin-5-one (**18**) and not the angular tricyclic (**18a**). Accordingly, the regioselective reaction of **1** with 2-benzylidenedimedone (**17**) in ethanol/acetic acid mixture, or by multicomponent condensation reaction of **1** with dimedone and benzaladehyde in ethanol/acetic acid mixture gave **18** [19a]. The objective of this work was directed to annulate **1** via one-step cyclocondensation reaction with **19** which leads to regioselective linear tetracyclic derivative **20**. Thus, Michael addition of **1** to 2-benzylidene indandione (**19**) in ethanol/acetic acid mixture afforded 3-methyl-4-phenyl-4,10-dihydroisoxazolo[4′,5′:5,6]pyrido[2,3-*a*]inden-5-one (**20**) in high yield and not the angular tetracyclic derivative **20a** (Scheme 4). Similarly, multicomponent condensation of **1** with indandione and benzaldehyde led to dihydropyrido[2,3-*a*]inden-5-one derivative **20**.

It is worthy to mention that the dihydropyridine derivative **20** is easily accessed via Hantzsch reaction and does not undergo auto-oxidation. Alternatively, Tu et al. [12, 15] obtained the annulated isoxazolo[5,4-*b*]pyridines through a feasible economic strategy by one-pot tandem reaction under microwave irradiation in water.

Linear heterocyclic annulation of isoxazolo[5,4-*b*]pyridine system through fusion to the pyridine moiety of the system was achieved through cycloaddition of **1** with α,β-unsaturated heterocycles. Compounds containing quinuclidine ring



Scheme 4. The pyridine ring formation through reaction of **1** with 2,6-dibenzylidenecyclohexanone and multicomponent reaction.

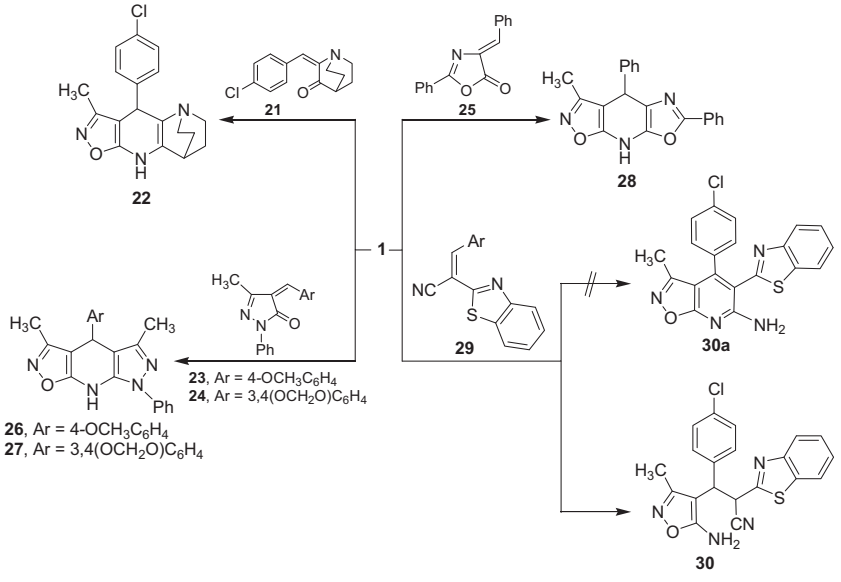
were reported to exhibit pharmacological activity and this enthused us to synthesize fused isoxazole moiety bearing a quinuclidine nucleus [19b]. Thus, Michael addition of **1** with *p*-chlorobenzylidene-3-quinuclidinone (**21**) in ethanol/acetic acid mixture led to the formation of **22** (Scheme 5). No attention has been paid to the similar reaction with benzylidenepyrazolone derivatives **23** and **24** or benzylideneoxazolone derivative **25** which can be used as a key intermediate for building of a pyridoisoxazole moiety fused to pyrazole or oxazole nucleus (**26**–**28**, respectively). Hence, the reaction of **1** with **23**, **24**, or **25** in ethanol/glacial acetic acid mixture gave the linear tricyclic derivatives **26**–**28**, respectively. Moreover, when compound **1** was reacted with 2-(benzo[*d*]thiazol-2-yl)-3-(4-chlorophenyl) acrylonitrile (**29**), a Michael adduct **30** was obtained instead of the fused isoxazolo[5,4-*b*]pyridine derivative **30a**.

Assignment of the newly synthesized compounds was based on elemental analyses, IR, ^{1}H NMR, and mass spectral data (cf. Experimental Section).

Biological activity

Effect of drugs on the viability of Ehrlich ascites carcinoma cells (EAC) in vitro

Thirteen oxazolopyridines and its annulated analogues were tested for potential antitumor activity against EAC *in vitro* [20]. Results for the IC_{100}, IC_{50}, and IC_{25} values of the active compounds are summarized in Table 1. The data showed clearly that all the tested compounds showed more toxicity



Scheme 5. Reaction of **1** with arylidenes of different heterocyclic compounds.

Table 1. *In vitro* potential antitumor activity of isoxazole analogues using EAC assay

Compound no.	% Dead		
	IC_{100} (mM)	IC_{50} (mM)	IC_{25} (mM)
5-FU	0.74	0.52	0.33
5	0.32	0.18	0.11
7	0.23	0.12	0.05
9	0.26	0.14	0.07
11	0.30	0.16	0.099
13	0.07	0.04	0.02
14	0.49	0.25	0.13
16	0.21	0.11	0.06
20	0.32	0.28	0.22
22	0.23	0.12	0.07
26	0.19	0.099	0.04
27	0.20	0.10	0.06
28	0.24	0.13	0.08
30	0.198	0.11	0.066

IC_{100}, IC_{50}, and IC_{25} are the inhibitive concentration at 25, 50, and 100 mM, respectively, of the compounds used. The dead % refers to the % of the dead tumor cells and 5-FU is 5-fluorouracil as a well-known cytotoxic agent.

than the 5-FU *in vitro* studies. Interestingly, compounds **13**, **26**, and **30** were around 5–10 times more toxic than the 5-FU. Experimental potential antitumor activity of the compounds reported in this study to their structures, the following structure activity relationships (SAR's) were postulated: (a) Compound **13** contains Meldrum's acid moiety and this is in agreement with that reported by Lukevics et al. [21]. (b) Also, in case of compound **26**, the high cytotoxic activity may be attributed to the presence of the pyrazole moiety which is in agreement with that reported by Perchellet et al. [22]. (c) Moreover, the high cytotoxic activity of compound **30** may be attributed to the presence of a benzothiazole moiety, similar results have been reported by Kok et al. [23] (Fig. 1).

Conclusions

Modification of oxazole derivatives produced compounds with potential for further development as anticancer agents. Based on these preliminary screening results, compound **13** showed significant activities in certain cancer cells and have been targeted for further studies. Additional research, including mode of action studies, is planned to accurately establish relative activity for SARs and rational design. Recently, the cancer chemopreventive effects of oxazole derivatives have been intensively investigated. Oxazole derivatives exhibited pronounced antitumor activities by triggering apoptosis in human tumor cells [24]. Studies are underway to investigate the apoptosis-inducing activity of compounds found to be cytotoxic in this study.

Experimental

All melting points are in degree centigrade (uncorrected) and were determined on Gallenkamp electric melting point apparatus. Elemental analyses were carried out at the Micro Analytical Center, Faculty of Science, Cairo University. IR spectra were recorded (KBr), (ν cm^{-1}) on a Mattson 5000 FTIR spectrophotometer at the Micro Analytical Center Faculty of Science, Mansoura University. The ^{1}H-NMR* spectra were measured on a Varian spectrophotometer at 300 MHz, using TMS as an internal reference and DMSO-d_6 or $CDCl_3$ as solvent at the Chemistry Department, Faculty of Science, Cairo University. The ^{1}H-NMR** and ^{13}C-NMR spectra were acquired on a JEOL ECX-400 spectrometer at the Chemistry Department, School of Engineering and Science, Jacobs University of Bremen, Germany, operating at 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR at room temperature in $CDCl_3$, and DMSO-d_6 using a 5 mm probe. The chemical shifts (δ) are reported in parts per million and where referenced to the residual solvent peak. High resolution mass spectra (HRMS) were recorded using both a Bruker HCT ultra and a high resolution (Bruker Daltonics micrOTOF) instrument from methanol or dichloromethane solutions using the positive electrospray ionization mode (ESI). The ^{1}H-NMR*** spectra were measured on a Varian spectrophotometer at 500 MHz, using TMS as an internal reference and $CDCl_3$ as solvent at the National Research Center, Cairo. Mass spectra were recorded on (Kratos, 70 eV) MS equipment and/or a Varian MAT 311 A spectrometer, at the Microanalytical Center, Faculty of Science, Cairo University. Reaction mixtures were monitored by thin layer chromatography (TLC) using EM science silica gel coated plates with visualization by irradiation with ultraviolet lamp. Biological testing was carried out at the Drug Department, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

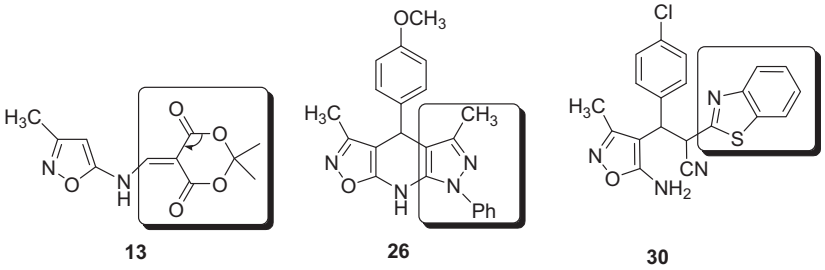


Figure 1. Structures of the most potent compounds **13**, **26**, and **30**.

Reaction of 1 with α,β-unsaturated ketones

General procedure

A mixture of 5-amino-3-methylisoxazole (**1**) (0.40 g, 4 mmol) and α,β-unsaturated ketones namely; (*E*)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (**2**) (0.95 g, 4 mmol), (*E*)-4-(4-methoxyphenyl)-but-3-en-2-one (**4**) (0.7 g, 4 mmol) or 1,3-dibenzalacetone (**6**) (0.94 g, 4 mmol) in dimethylformamide (25 mL) was refluxed for appropriate time and then left to cool. Ethanol (25 mL) was added to the reaction mixture, then left in a refrigerator overnight. The formed precipitate was filtered and recrystallized from the appropriate solvents to give the corresponding pyridine derivatives **3**, **5**, and **7**.

*4-(4-Methoxyphenyl)-3-methyl-6-phenylisoxazolo[5,4-b]-pyridine (**3**)*

Reaction time 15 h; yield 60%; yellow crystals; m.p. 199–200°C (ethanol); $R_f = 0.4$ [pet. ether (40–60): ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 2967, 2933$ (CH, str.), 1606 (C=C); ^{1}H-NMR* (DMSO-d_6) δ (ppm): 2.31 (s, 3H, CH_3), 3.86 (s, 3H, OCH_3), 7.13–8.27 (m, 10H, aromatic-CH and pyridine-CH=C); HRMS(micrOTOF): *m/z* for $C_{20}H_{16}N_2O_2$, Calcd.: 316.3500. Found: 317.0000 (M^++1), 339.0000 (M^++Na), 655.0000 ($2M^+$+Na); MS (EI, 70 eV) *m/z* (%) = 317 (M^++H, 24.7), 316 (M^+, 100.0), 315 (M^+−1, 10.9), 301 (3.1), 288 (13.4), 285 (4.0), 273 (38.3), 239 (1.4), 209 (0.3), 177 (7.6), 107 (4).

*4-(4-Methoxyphenyl)-3,6-dimethyl-4,7-dihydroisoxazolo-[5,4-b]pyridine (**5**)*

Reaction time 10 h; yield 40%; yellow crystals; m.p. 299–300°C (DMF); $R_f = 0.5$ [pet. ether (40–60)/ethyl acetate (1:4)]; IR (KBr): $\nu/cm^{-1} = 3409, 3369$ (NH), 1664, 1610 (C=C), (C=N); ^{1}H-NMR* (DMSO-d_6) δ (ppm): 2.29 (s, 3H, CH_3), 2.60 (s, 3H, CH_3), 3.76 (s, 3H, OCH_3), 7.50 (d, $J = 8.6$ Hz, 2H, aromatic-CH) and 7.70 (d, $J = 8.4$ Hz, 2H, aromatic); HRMS(micrOTOF): *m/z* for ($C_{15}H_{14}N_2O_2$+H), Calcd.: 255.1134. Found: 255.2000 (M^++H).

*3-Methyl-4-phenyl-6-styrylisoxazolo[5,4-b]pyridine (**7**)*

Reaction time 10 h, yield 50%; yellow crystals; m.p. 270–271°C (ethanol); $R_f = 0.3$ [pet. ether (40–60)/ethyl acetate (1:4)]; IR (KBr): $\nu/cm^{-1} = 3409$ (NH), 2969, 2929 (CH, str.), 1644 (C=N), 1600 (C=C); ^{1}H-NMR* (DMSO-d_6) δ (ppm): 2.5 (s, 3H, CH_3), 6.55 (d, $J = 5.4$ Hz, 1H, Ph-CH=CH), 6.60 (d, $J = 5.6$ Hz, 1H, Ph-CH=CH), 7.16–8.07 (m, 11H, aromatic-CH and pyridine-CH=C); MS (EI, 70 eV) *m/z* (%) = 312 (M^+, 2.1), 324 (4.6), 236 (7.1), 132 (13.4), 131 (100.0), 130 (12.6), 104 (26.8), 103 (61.5), 102 (17.6), 91 (10), 77 (47.3), 51 (16.3). Anal. Calcd. for $C_{21}H_{16}N_2O$ (312.37): C, 80.75; H, 5.16%. Found: C, 80.83; H, 5.22%.

*4-(4-Methoxyphenyl)-3-methylisoxazolo[5,4-b]pyridine-6-carboxylic acid (**9**)*

A mixture of **1** (0.49 g, 5 mmol) and (*E*)-4-(4-methoxyphenyl)-2-oxobut-3-enoic acid (**8**) [25], (1.03 g, 5 mmol) in glacial acetic acid (5 mL) was refluxed for 30 min, then allowed to cool, ethanol (10 mL) was added, and the reaction mixture was allowed to stand overnight. The precipitate formed was filtered and recrystallized from ethanol to give **9**.

Yield 80%; gray crystals; m.p. 218°C (ethanol); $R_f = 0.3$ [pet. ether (40–60): ethyl acetate (1:3); IR (KBr): $\nu/cm^{-1} = 3434$ (OH), 2840, 2939 (CH, str.), 1677 (CO), 1598 (C=C); ^{1}H-NMR* (DMSO-d_6) δ (ppm): 2.32 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 6.77 (d, $J = 8.2$ Hz, 2H, aromatic-CH), 6.9 (d, $J = 8.3$ Hz, 2H, aromatic-CH), 8.0 (s, 1H, pyridine-CH=C), 13.0 (s, 1H, COOH); HRMS (micrOTOF): *m/z* for $C_{15}H_{12}N_2O_4$, Calcd.: 284.2700. Found: 285.1000 (M^++H), 307.0000 [M^++Na]; MS (EI, 70 eV) *m/z* (%) = 285 (M^++H, 12.4), 284 (M^+, 100.0), 283 (M^+−H, 7.8), 239 (7.8), 132 (12.4), 117 (10.9), 107 (8.5).

*4-(4-Methoxyphenyl)-3-methyl-4,5-dihydroisoxazolo[5,4-b]-pyridin-6(7H)-one (**11**)*

A mixture of **1** (0.49 g, 5 mmol) and (1.30 g, 5 mmol) of *p*-anisylidene Meldrum's acid [26], (**10**) in dry nitrobenzene (5 mL) was heated under reflux for 30 min, left to cool, the formed precipitate was filtered off, washed with ethanol, and recrystallized from ethanol to afford **11**.

Yield 57%; yellow crystals; m.p.190°C (ethanol); $R_f = 0.5$ [pet. ether (40–60): ethyl acetate (2:5)]; IR (KBr): $\nu/cm^{-1} = 3430$ (NH), 1720 (CO), 1644, 1622 (C–N), (C=C); ^{1}H-NMR*** ($CDCl_3$) δ (ppm): 2.31 (s, 3H, CH_3), 3.04 (dd, $J_{a,x} = 9.2$ Hz, 1H, 5-H_a), 3.18 (dd, $J_{a,b} = 5.4$ Hz, 1H, 5-H_b), 3.6 (t, 1H, 4-H_x), 3.77 (s, 3H, OCH_3), 6.83 (d, $J = 8.4$ Hz, 2H, aromatic-CH), 6.99 (d, $J = 8.5$ Hz, 2H, aromatic-CH), 8.07 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 260 (M^++2, 2.3), 259 (M^++H, 11.8), 258 (M^+, 43.4), 243 (15.6), 215 (70.3), 217 (11.7), 151 (4.2), 148 (13.5), 147 (100.0), 135 (8.4), 107 (4.4). Anal. Calcd. for $C_{14}H_{14}N_2O_3$ (258.28): C, 65.11; H, 5.46%. Found: C, 65.33; H, 5.54%.

*2,2-Dimethyl-5-[(3-methylisoxazol-5-ylamino)methylene]-1,3-dioxane-4,6-dione (**13**)*

A mixture of Meldrum's acid (**12**) (0.72 g, 5 mmol) and methyl orthoformate (25 mmol) was refluxed for 2.5 h, and then (0.49 g, 5 mmol) of **1** was added. The reaction mixture was heated for 15 min. The formed precipitate was filtered off and recrystallized from EtOH/DMF (1:1) to furnish **13**.

Yield 60%; yellow crystals; m.p. 203°C [EtOH-DMF (1:1)]; $R_f = 0.4$ [pet. ether (40–60): ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 3294$ (NH), 2925 (CH, str.), 1743, 1693 (2C=O), 1643 (C=N), 1609 (C=C); ^{1}H-NMR* ($CDCl_3$) δ (ppm): 1.75 (s, 6H, $2CH_3$), 2.29 (s, 3H, CH_3), 5.7(s, 1H, isoxazole-CH), 7.20 (s, 1H, NH), 8.53 (s, 1H, C=CH); MS (EI, 70 eV) *m/z* (%) = 252 (M^++H, 5.3), 251 (M^+, 20.0), 194 (28.3), 193 (74.7), 108 (91.9), 81 (56.6), 80 (100.0), 54 (37.9), 52 (58.7). Anal. Calcd. for $C_{11}H_{12}N_2O_5$ (252.07): C, 52.38; H, 4.80%. Found: C, 52.25; H, 4.675%.

*3-Methyl-4,7-dihydroisoxazolo[5,4-b]pyridin-4-one (**14**)*

Compound **13** (1.26 g, 5 mmol) in nitrobenzene was refluxed for 30 min. The formed precipitate after cooling was filtered, washed with ethanol, and crystallized from ethanol/DMF (1:1) to afford **14**.

Yield 77%; yellow crystals; m.p. 203°C [EtOH-DMF (1:1)]; $R_f = 0.5$ [pet. ether (40–60): ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 3294$ (NH), 2983, 2933 (CH, str.), 1769 (C=O), 1633 (C=N), 1610 (C=C); ^{1}H-NMR* ($CDCl_3$) δ (ppm): 2.63 (s, 3H, CH_3), 6.70 (s, 1H, NH), 7.58 (d, 1H, $CH_5 = CH_6$), 8.26 (d, 1H, $CH_5 = CH_6$); MS (EI, 70 eV) *m/z* (%) = 151 (M^++H,

5.3), 150 (M^+, 20.0), 122 (5.6), 97 (14.7), 81 (60.6), 80 (100.0). Anal. Calcd. for $C_7H_6N_2O_2$ (150.14): C, 56.00; H, 4.03%. Found: C, 56.18; H, 4.11%.

*(E)-8-Benzylidene-3-methyl-4-phenyl-4,5,6,7,8,9-hexahydroisoxazolo[5,4-b] quinoline (**16**)*

A mixture of **1** (0.40 g, 4 mmol) and 2,6-dibenzalcyclohexanone (**15**) (4 mmol) in dimethylformamide (25 mL) was refluxed for 10 h, then left to cool. Ethanol (25 mL) was added to the reaction mixture then left in a refrigerator overnight. The formed precipitate was filtered and recrystallization from ethanol gave **16**.

Yield 40%; yellow crystals; m.p. 270–272°C (ethanol); $R_f = 0.3$ [pet. ether (40–60)/ethyl acetate (1:4)]; IR (KBr): $\nu/cm^{-1} = 3370$ (NH), 2923, 2852 (CH, str.), 1654, 1611 (C=N), (C=C), ^{1}H-NMR* ($CDCl_3$) δ (ppm): 2.53 (s, 3H, CH_3), 2.72–2.93 (m, 6H, 3 CH_2), 7.4 (s, 1H, CH=C), 7.50–7.91(m, 10H, aromatic CH); MS (EI, 70 eV) *m/z* (%) = 354 (M^++2, 2.3), 352 (M^+, 2.7), 264 (2.67), 194 (16.6), 193 (73.0), 177 (10.7), 176 (17.9), 105 (12.2), 104 (11.7), 98 (3.2), 80 (14.9), 44 (100.0). Anal. Calcd. for $C_{24}H_{20}N_2O$ (352.43): C, 81.79; H, 5,72%%; Found: C, 81.52; H, 5.48%.

*General procedure for preparation of **18** and **20***

Method (A)

A mixture of **1** (0. 49 g, 5 mmol) and 2-benzylidenedimedone (**17**) [25] or 2-benzylideneindandione (**19**) (5 mmol) in ethanol (50 mL) and glacial acetic acid (1 mL) was refluxed for 3 h, the reaction mixture was allowed to stand overnight. The precipitate formed was filtered and recrystallized from the proper solvent to give **18** and **20**, respectively.

Method (B)

A mixture of **1** (0.49 g, 5 mmol), dimedone (0.7 g, 5 mmol) or indandione (0.73 g, 5 mmol) and benzaldehyde (0.5 mL, 5 mmol) in ethanol (15 mL) and glacial acetic acid (1 mL) was heated under reflux for 3 h. The solvent was evaporated under vacuum to give yellow precipitate, which was washed with water then recrystallized from proper solvent to give **18** and **20**, respectively.

*3,7,7-Trimethyl-4-phenyl-4,6,8,9-tetrahydroisoxazolo-[5,4-b]quinolin-5-one (**18**)*

Yield 60% [A]; 88% [B]; yellow crystals; m.p. 222–224°C (benzene); $R_f = 0.5$ [pet. ether (40–60): ethyl acetate (3:8)]; IR (KBr): $\nu/cm^{-1} = 3207$ (NH), 2958, 2931, 2877 (CH, str.), 1670 (CO), 1610 (C=C); ^{1}H-NMR*** ($CDCl_3$) δ (ppm): 1.9 (s, 6H, $2CH_3$), 2.1 (s, 2H, 8-CH_2), 2.16–2.2 (dd, 2H, 6-CH_2), 2.4 (s, 3H, CH_3), 5.05 (s, 1H, 4-H), 7.2 (s, 1H, 9-NH), 7.22–7.25 (m, 5H, aromatic-CH); HRMS (micrOTOF): *m/z* for $C_{19}H_{20}N_2O_2$, Calcd.: 308.3700. Found: 307.0000 (M^+ − H), 331.1000 (M^++Na), 639.3000 ($2M^+$+Na); MS (EI, 70 eV) *m/z* (%) = 308 (M^+, 20.6), 307 (M^+−H, 19.5), 234 (2.1), 293 (10.1), 251 (3.3), 239 (4.4), 231 (100.0).

*3-Methyl-4-phenyl-4,10-dihydroisoxazolo[4′,5′:5,6]pyrido-[2,3-a]inden-5-one (**20**)*

Yield 90% [A]; 95% [B]; red crystals; m.p. 261–263°C (DMF); $R_f = 0.6$ [pet. ether (40–60)/ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 3340$ (NH), 2926 (CH, str.), 1719 (C=O), 1594 (C=C); ^{1}H-NMR** (DMSO-d_6) δ (ppm): 1.8 (s, 3H, CH_3), 4.9 (s, 1H, H-4), 7.22–7.51 (m, 9H, aromatic-CH), 12.02 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ (ppm): 10.48 (CH_3), 39.63 (C-4), 97.97 (C-4a), 108.82 (C-3a), 119.79, 121.06 (C-9), 121.41 (C-7), 127.16, 128.85, 130.92 (C-6), 132.50, 133.94 (C-8), 133.95, 144.84 (C-9a), 155.76 (C-3),159.65 (C-6a), 160 (C-1a), 190.94 (C=O); HRMS (micrOTOF): *m/z* for $C_{20}H_{13}N_2O_2$, Calcd.: 314.1100. Found: 313.0975 (M^+−H); MS (EI, 70 eV) *m/z* (%) = 315 (M^++H, 0.3), 314 (M^+, 2.7), 313 (M^+−H, 29.3), 312 (M^+−2, 100.0), 311 (38.7), 297 (9.3), 217 (3.0), 124 (8.3), 123 (7.3), 97 (5.7), 98 (17.0).

*Reaction of **1** with α,β-unsaturated carbonyl compounds*

A mixture of **1** (0. 49 g, 5 mmol), α,β-unsaturated carbonyl derivatives namely; *p*-chlorobenzylidene-3-quinuclidinone (**21**) (1.24 g, 5 mmol), **23** (1.46 g, 5 mmol), **24** (1.53 g, 5 mmol) or **25** (1.25 g, 5 mmol) in ethanol (50 mL) and glacial acetic acid (20 mL) was refluxed for the necessary time. The reaction mixture was allowed to stand overnight at room temperature to give the linear fused tricyclic systems **22** and **26–28**, respectively.

*3-Methyl-4-(4-chlorophenyl)-4,11-dihydrooxazolo-[4′,5′:5,6]pyrido[2,3-b]quinuclidine (**22**)*

Reaction time 5 h, yield 60%; brown crystals; m.p. 256–257°C (DMF); $R_f = 0.6$ [pet. ether (40–60): ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 3439$ (NH), 2954 (CH, str.), 1605 (C=C); ^{1}H-NMR* (DMSO-d_6) δ (ppm): 1.57–1.98 (m, 4H, $(CH_2)_2$–C), 2.68 (s, 3H, CH_3), 3.15 (m, 4H, $(CH_2)_2$–N), 3.22 (q, 1H, bridgehead), 3.74 (s, 1H, NH), 7.53 (d, $J = 8.1$ Hz, 2H, aromatic-CH), 8.12 (d, $J = 8.1$ Hz, 2H, aromatic-CH); MS (EI, 70 eV) *m/z* (%) = 327/329 (M^+, 28.3:9.2), 214 (6.3), 137 (36.6), 125 (19.5), 111 (22.4), 76 (43.9), 51 (100.0). Anal. Calcd. for $C_{18}H_{18}ClN_3O$ (327.82): C, 65.95; H, 5.53%. Found: C, 65.68; H, 5.70%.

*4-(4-Methoxyphenyl)-3,5-dimethyl-7-phenyl-4a,7-dihydro-4H-pyrazolo [4′,3′:5,6]pyrido[3,2-d]isoxazole (**26**)*

Reaction time 4 h, yield 40%; white crystals; m.p. 220°C (ethanol); $R_f = 0.6$ [pet. ether (40–60)/ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 3300$ (NH), 3000, 2990 (CH, str.); ^{1}H-NMR* (DMSO-d_6) δ (ppm): 2.19 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.61 (s, 3H, OCH_3), 5.04 (s, 1H, CH), 6.74 (d, $J = 8.6$ Hz, 2H, aromatic-CH), 6.78 (d, $J = 9.$ 2 Hz, 2H, aromatic-CH)7.04–7.28 (m,5H, aromatic-CH), 11.38 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 373 (M^++H, 0.3), 372 (M^+, 0.6), 292 (10.0), 158 (1.0), 116 (6.8), 92 (8.1), 82 (18.7), 77 (100.0). Anal. Calcd. for $C_{22}H_{20}N_4O_2$ (372.43): C, 70.95; H, 5.41%. Found: C, 70.69; H, 5.56%.

*4-(Benzo[d][1,3]dioxol-5-yl)-3,5-dimethyl-7-phenyl-7,8-dihydro-4H-pyrazolo [4′,3′:5,6]pyrido[3,2-d]isoxazole (**27**)*

Reaction time 3 h, yield 50%; white crystals; m.p. 229°C (ethanol); $R_f = 0.6$ [pet. ether (40–60): ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 3320$ (NH), 2997, 2995 CH, str.); ^{1}H-NMR* (DMSO-d_6) δ (ppm): 2.19 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 4.74 (s, 1H, CH), 5.92 (s, 2H, O–CH_2–O), 6.67–7.66 (m, 8H, aromatic-CH), 11.34 (s, 1H, NH); MS (EI, 70 eV) *m/z* (%) = 387 (M^++H, 3.65), 386 (M^+, 71.1), 359 (52.0), 356 (47.7),

266 (45.0) 307 (3.9), 124 (55.6), 105 (100.0), 99 (65.9). Anal. Calcd. for $C_{22}H_{18}N_4O_3$ (386.41): C, 68.38; H, 4.70%. Found: C, 68.51; H, 4.62%.

*1-Methyl-6-phenyl-8-phenyl-7,8-dihydro-oxazolo-[4′,5′:5,6]pyrido[3,2-d]isoxazole (**28**)*

Reaction time 5 h, yield 70%; white crystals; m.p. 145°C (ethanol); $R_f = 0.6$ [pet. ether (40–60): ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 3264$ (NH), 1643 (C=N); ^{1}H-NMR** ($CDCl_3$) δ (ppm): 2.50 (s, 3H, CH_3), 4.20 (s, 1H, CH), 7.35–8.02 (m, 10H, aromatic), 10.12 (s, 1H, NH). Anal. Calcd. for $C_{20}H_{15}N_3O_2$ (329.36): C, 72.94; H, 4.59%. Found: C, 72.76; H, 4.63%.

*3-(5-Amino-3-methylisoxazol-4-yl)-2-(benzo[d]thiazol-2-yl)-3-(4-chlorophenyl) propiononitrile (**30**)*

A mixture of **1** (0.49 g, 5 mmol), 2-(benzo[*d*]thiazol-2-yl)-3-(4-chlorophenyl) acrylonitrile (**29**) (1.50 g, 5 mmol) was refluxed for 3 h in ethanol (50 mL) and triethylamine (few drops) for 3 h, then allowed to stand overnight at room temperature. The formed precipitate was collected by filtration to give **30**.

Yield 55%; white crystals; m.p. 240°C (ethanol); $R_f = 0.6$ [pet. ether (40–60): ethyl acetate (1:3)]; IR (KBr): $\nu/cm^{-1} = 3392$ (NH_2), 3061, 2926 (CH, str.), 2261 (CN), 1632 (C=N), 1594 (C=C); ^{1}H-NMR* ($CDCl_3$) δ (ppm): 1.86 (s, 3H, CH_3), 5.40 (s, 2H, NH_2), 7.08 (d, $J = 6.1$ Hz, 2H, aromatic-CH), 7.26 (d, $J = 6.5$ Hz, 2H, aromatic-CH), 7.59 (d, $J = 6.1$ Hz, 2H, aromatic-CH), 8.12 (d, $J = 7.8$ Hz, 2H, aromatic-CH), 8.22 (d, $J = 8.1$ Hz, 2H, aromatic-CH); MS (EI, 70 eV) *m/z* (%) = 394/396 (M^+, 35.1/11.0), 134 (6.5), 111 (8.3), 69 (100.0), 66 (14.9). Anal. Calcd. for $C_{20}H_{15}ClN_4OS$ (394.88): C, 60.83; H, 3.83%. Found: C, 60.96; H, 3.68%.

Antitumor activity

Cytotoxic evaluation of the tested compounds

EAC were obtained from National Cancer Institute, Cairo, Egypt. To examine whether the synthesized compounds have a direct cytotoxic effect on EAC [20], viability and the percentage of viable cells was estimated by the trypan blue [27] exclusion test. The desired concentration of tumor cells (2×10^6 cells per 0.2 mL) was obtained by dilution with saline solution (0.9% sodium chloride). The viability of the tumor cells obtained and used in this experiment was always higher than 90%. Below this percentage, the cells were discarded and the entire procedure was repeated for three times.

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