Anti-inflammatory Activity and Acute Toxicity (LD_{50}) of Some New Synthesized Pyridin-2-yl)phenyl)-2-methoxybenzamide and Thieno[2,3-b]pyridine Derivatives

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Abstract: In continuation of our previous work, a series of substituted pyridine derivatives **(3-12)** were synthesized according to our previous reported procedures using chalcone derivatives **2a-c** as starting materials. The pharmacological screening showed that many of these obtained compounds have good anti-inflammatory activities comparable to Prednisolone® as a reference drug. Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50}. The structures of newly synthesized compounds were confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS spectral data and elemental analysis. The detailed synthesis, spectroscopic data, LD_{50} and pharmacological activities of the synthesized compounds were reported.

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1. Introduction

In a previous work, we reported that substituted heterocyclic derivatives act as analgesic, anticonvulsant, and antiandrogenic agents [Amr et al., 2003 a; Amr et al., 2006; Amr et al., 2005; Amr, 2005], and their antimicrobial activity [Amr et al., 1999; Attia et al., 1997; Attia et al., 2000 a ; Attia et al., 2000 b]. In addition, the androgenic, anabolic, and anti-inflammatory activities of many heterocyclic steroidal derivatives have been reported [Amr and Abdulla, 2002]. On the other hand, cyanopyridone and cyanopyridine derivatives have promising antimicrobial agents [Amr, 2000; Amr et al., 2003b], as well as anticancer activities [Hammam et al., 2003; Hammam et al., 2000; Hammam et al., 2001; Hammam et al., 1996; Hammam et al., 1997]. Recently, some new heterocyclic compounds containing pyridine moiety have been reported as anticancer and anti-inflammatory agents [Khalifa et al., 2013; Al-Harbi et al., 2013]. Synthesis of the pyridine ring system and its derivatives occupy an important place in the realm of synthetic organic chemistry, due to their therapeutic and pharmacological properties [Henry, 2004; Bagley et al., 2005; Gilchrist, 2001]. They have emerged as integral backbones of over 7000 existing drugs [Li et al., 1999; Vacher et al., 1999]. The pyridine ring is also an integral part of anticancer and anti-inflammatory agents [Son et al., 2008; Amr and Abdulla 2006]. Pyridin-2(1*H*)-ones are known to possess a range of biological activities such as analgesic, antifungal, antimalarial, antibacterial, anti-HIV, phytotoxic, antitumoral and antiviral properties [Öztürk et al., 2001; Findlay et al., 1978; Storck et al., 2005; Macdonald et al., 2008; Evidente et al., 2006; Cocco et al., 2000; 2003; Al-Abdullah, 2011]. In view of these observations and in continuation of our previous work in pyridine chemistry, we synthesized some new heterocyclic compounds containing pyridine, thiopyridone, pyridine rings and tested their pharmacological screening.

2. Experimental

2.1. Chemistry

Melting points are uncorrected and determined with electro thermal capillary apparatus and were uncorrected. Elemental analyses were performed in the Microanalytical Unit, Faculty of Science, Ain Shams University using Perkin Elmer CHN 2400 (one Run), Egypt and were found within ±0.4% of the theoretical values. The IR spectra were recorded in (KBr) on a Shimadzu CVT-04 spectrophotometer. The ^{1}H- and ^{13}C NMR spectra were determined on Varian Gemini 270 MHz usng $CDCl_3$ or DMSO-d_6 as solvent using TMS as an internal standard. The mass spectra were performed using a Varian MAT CH-5 spectrometer (70 eV). All reactions were followed by TLC (silica gel, aluminum sheets 60F_{254}, Merck).

2.1a Synthesis of N-(4-(4-(aryl)-5-cyano-6-oxo-6H-pyran-2-yl)phenyl)-5-chloro-2-methoxy-benzamide 3a-c

A mixture of **2a-c** (2 mmol), ethyl cyanoacetate (0.26 ml, 2.4 mmol) and sodium ethoxide (0.136g, 2 mmol) in absolute ethanol (20 ml) was refluxed for 2hrs. The reaction mixture was evaporated under reduced pressure; the residue was solidified with water, filtered off, dried and crystallized from benzene/methanol to give the corresponding compounds **3a-c**, respectively.

5-Chloro-N-(4-(5-cyano-6-oxo-4-phenyl-6H-pyran-2-yl)phenyl)-2-methoxybenzamide (3a):

Yield 56%, mp. 236-238°C; IR (KBr, cm^{-1}) ν: 3478 (NH), 1742 (CO), 2223 (CN), 1668 (amide I), 1618 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 3.48 (s, 3H, OCH_3), 7.16 (s, 1H, pyran-H), 7.08-7.85 (m, 12H, Ar-H), 11.05 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 56.35 (OCH_3), 118.45 (CN), 164.05 (CONH), 156.96, 154.35, 145.64, 131.28, 107.88 (5C, pyran-C), 133.23, 126.85, 133.02, 128.40, 114.72, 158.90, 138.68, 117.36, 129.50, 137.61, 136.14, 123.40, 126.80, 132.01 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 456 [M^+]. Elemental analysis for $C_{26}H_{17}ClN_2O_4$ (456.88): Calcd. C, 68.35; H, 3.75; Cl, 7.76; N, 6.13. Found: C, 68.30; H, 3.70; Cl, 7.70; N, 6.10.

N-(4-(4-(4-Bromophenyl)-5-cyano-6-oxo-6H-pyran-2-yl)phenyl)-5-chloro-2-methoxybenzamide (3b):

Yield 63%, mp. 254-256°C; IR (KBr, cm^{-1}) ν: 3472 (NH), 1746 (CO), 2220 (CN), 1665 (amide I), 1622 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.39 (s, 3H, OCH_3), 7.12 (s, 1H, pyran-H), 7.30-7.92 (m, 11H, Ar-H), 10.95 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.71 (OCH_3), 117.54 (CN), 163.75 (CONH), 157.89, 154.70, 145.60, 131.18, 107.90 (5C, pyran-C), 134.09, 127.55, 133.90, 128.15, 115.50, 153.17, 139.90, 117.88, 129.84, 144.91, 144.83, 129.47, 131.90, 123.50 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 536 [M^+]. Elemental analysis for $C_{26}H_{16}BrClN_2O_4$ (535.77): Calcd. C, 58.29; H, 3.01; Cl, 6.62; N, 5.23. Found: C, 58.25; H, 2.96; Cl, 6.58; N, 5.20.

5-Chloro-N-(4-(4-(2-chlorophenyl)-5-cyano-6-oxo-6H-pyran-2-yl)phenyl)-2-methoxybenzamide (3c):

Yield 68%, mp. 282-284°C; IR (KBr, cm^{-1}) ν: 3478 (NH), 1742 (CO), 2223 (CN), 1688 (amide I), 1618 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 3.42 (s, 3H, OCH_3), 7.18 (s, 1H, pyran-H), 7.32-7.96 (m, 11H, Ar-H), 11.12 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 56.66 (OCH_3), 117.46 (CN), 164.05 (CONH), 157.92, 154.65, 145.68, 131.35, 107.85 (5C, pyran-C), 122.64, 134.14, 127.50, 126.65, 133.92, 128.32, 115.64, 153.24, 139.78, 117.94, 129.80, 144.94, 145.16, 129.64, 131.67, 123.57 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 491 [M^+]. Elemental analysis for $C_{26}H_{16}Cl_2N_2O_4$(491.32): Calcd. C, 63.56; H, 3.28; Cl, 14.43; N, 5.70. Found: C, 63.50; H, 3.22; Cl, 14.38; N, 5.65.

2.1b Synthesis of N-(4-(4-(aryl)-5-cyano-1,6-dihydro-6-oxopyridin-2-yl)phenyl)-5-chloro-2-methoxybenzamide 4a-c

Method A:

A mixture of **2a-c** (2 mmol), ethyl cyanoacetate (0.26 ml, 2.4 mmol) and ammonium acetate (1.24 g, 16 mmol) in n-butanol (20 ml) was refluxed for 2hrs. After cooling, the obtained precipitate was collected by filtration, washed with water, dried and crystallized from methanol to give the corresponding cyanopyridone derivatives **4a-c**, respectively.

Method B:

A solution of **3** (2 mmol), ethyl cyanoacetate (0.26 ml, 2.4 mmol), aromatic aldehydes (2 mmol) and ammonium acetate (1.24 g, 16 mmol) in n-butanol (20 ml) was refluxed for 2 hrs. After cooling, the obtained precipitate was collected by filtration, washed with water, dried and crystallized from methanol to give the corresponding cyanopyridone derivatives **4a-c**, respectively.

5-Chloro-N-(4-(5-cyano-1,6-dihydro-6-oxo-4-phenylpyridin-2-yl)phenyl)-2-methoxybenzamide (4a):

Yield 91% [A], 88% [B], mp. 237-239°C; IR (KBr, cm^{-1}) ν: 3528 (OH), 3415 (NH), 2251 (CN), 1670 (amide I), 1621 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.52 (s, 3H, OCH_3), 7.04 (s, 1H, pyrid-H), 7.12-8.05 (m, 12H, Ar-H), 8.12 (s, 1H, OH exchangeable with D_2O), 11.20 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.71 (OCH_3), 118.29 (CN), 165.71 (CONH), 163.75, 113.13, 142.53, 134.12, 162.42 (5C, pyrid-C), 134.81, 127.90, 133.35, 127.90, 112.80, 156.70, 141.80, 117.75, 130.87, 141.90, 141.51, 127.71, 128.90, 130.25 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 456 [M^+]. Elemental analysis for $C_{26}H_{18}ClN_3O_3$ (455.89): Calcd. C, 68.50; H, 3.98; Cl, 7.78; N, 9.22. Found: C, 68.42; H, 3.92; Cl, 7.72; N, 9.20.

N-(4-(4-(4-Bromophenyl)-5-cyano-1,6-dihydro-6-oxopyridin-2-yl)phenyl)-5-chloro-2-methoxybenzamide (4b):

Yield 90% [A], 78% [B], mp. 275-277°C; IR (KBr, cm^{-1}) ν: 3530 (OH), 3405 (NH), 2245 (CN), 1671 (amide I), 1621 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.58 (s, 3H, OCH_3), 7.10 (s, 1H, pyrid-H), 7.24-8.10 (m, 11H, Ar-H), 8.07 (s, 1H, OH exchangeable with D_2O), 10.98 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.75 (OCH_3), 118.36 (CN), 165.22 (CONH), 163.70, 113.18, 142.50, 134.18, 162.40 (5C, pyrid-C), 134.00, 127.62, 133.88, 128.32, 115.48, 153.10, 139.82, 118.05, 129.80, 144.53, 145.16, 129.56, 132.00, 123.66 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 535 [M^+]. Elemental analysis for $C_{26}H_{17}BrClN_3O_3$ (534.79): Calcd. C, 58.39; H, 3.20; Cl, 6.63; N, 7.86. Found: C, 58.33; H, 3.16; Cl, 6.60; N, 7.80.

5-Chloro-N-(4-(4-(2-chlorophenyl)-5-cyano-1,6-dihydro-6-oxopyridin-2-yl)phenyl)-2-methoxybenzamide (4c):

Yield 85% [A], 72% [B], mp. 254-256°C; IR (KBr, cm^{-1}) ν: 3516 (OH), 3451 (NH), 2248 (CN), 1668 (amide I), 1621 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 3.50 (s, 3H, OCH_3), 7.08 (s, 1H, pyrid-H), 7.28-7.95 (m, 11H, Ar-H), 8.12 (s, 1H, OH exchangeable with D_2O), 11.05 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 56.70 (OCH_3), 118.47 (CN), 165.18 (CONH), 163.64, 113.22, 142.65, 134.32, 162.52 (5C, pyrid-C), 122.60, 134.16, 127.47, 126.60, 133.90, 128.38, 115.60, 153.32, 139.70, 117.88, 129.82, 144.90, 145.24, 129.66, 131.62, 123.53 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 490 [M^+]. Elemental analysis for $C_{26}H_{17}Cl_2N_3O_3$ (490.34): Calcd. C, 63.69; H, 3.49; Cl, 14.46; N, 8.57. Found: C, 63.63; H, 3.44; Cl, 14.40; N, 8.50.

2.1c Synthesis of N-(4-(4-(aryl)-5-cyano-1,6-dihydro-6-thioxopyridin-2-yl)phenyl)-5-chloro-2-methoxybenzamide 5a-c

Method A:

A mixture of **2a-c** (2 mmol), thiocyanoacetamide (0.240 g, 2.4 mmol) and ammonium acetate (1.24 g, 16 mmol) in n-butanol (25 ml) was refluxed for 3 hrs. After cooling, the precipitated solid product was filtered off, washed with water, dried and crystallized from benzene to give the corresponding cyanothiopyridone derivatives **5a-c**, respectively.

Method B:

A solution of **3** (2 mmol), thiocyanoacetamide (0.240 ml, 2.4 mmol), aromatic aldehydes (2 mmol) and ammonium acetate (1.24 g, 16 mmol) in n-butanol (20 ml) was refluxed for 2 hrs. After cooling, the obtained precipitate was collected by filtration, washed with water, dried and crystallized from benzene to give the corresponding cyanopyridone derivatives **5a-c**, respectively.

5-Chloro-N-(4-(5-cyano-1,6-dihydro-4-phenyl-6-thioxopyridin-2-yl)phenyl)-2-methoxybenzamide (5a):

Yield 82%, mp. 218-220°C; IR (KBr, cm^{-1}) ν: 3531 (NH), 3415 (SH), 2232 (CN), 1672 (amide I), 1633 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 3.55 (s, 3H, OCH_3), 3.98 (s, 1H, SH), 7.12 (s, 1H, pyrid-H), 7.22-8.10 (m, 12H, Ar-H), 10.81 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 56.1 8 (OCH_3), 118.33 (CN), 164.56 (CONH), 163.58, 138.82, 145.50, 112.65, 171.04 (5C, pyrid-C), 134.74, 127.85, 133.42, 127.88, 112.74, 156.82, 141.66, 117.55, 130.83, 141.86, 141.55, 127.64, 128.66, 130.34 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 472 [M^+]. Elemental analysis for $C_{26}H_{18}ClN_3O_2S$ (471.96): Calcd. C, 66.17; H, 3.84; Cl, 7.51; N, 8.90; S, 6.79. Found: C, 66.12; H, 3.80; Cl, 7.44; N, 8.85; S, 6.71.

N-(4-(4-(4-Bromophenyl)-5-cyano-1,6-dihydro-6-thioxopyridin-2-yl)phenyl)-5-chloro-2-methoxy-benzamide (5b):

Yield 89%, mp. 217-219°C; IR (KBr, cm^{-1}) ν: 3508 (NH), 3388 (SH), 2256 (CN), 1671 (amide I), 1621 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.50 (s, 3H, OCH_3), 3.94 (s, 1H, SH), 7.16 (s, 1H, pyrid-H), 7.18-8.02 (m, 11H, Ar-H), 10.93 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.18 (OCH_3), 117.72 (CN), 163.85 (CONH), 163.56, 138.78, 145.58, 112.62, 171.14 (5C, pyrid-C), 134.05, 127.64, 133.90, 128.40, 115.50, 153.15, 139.84, 118.15, 129.76, 144.55, 145.22, 129.55, 132.04, 123.72 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 551 [M^+]. Elemental analysis for $C_{26}H_{17}BrClN_3O_2S$ (550.85): Calcd. C, 56.69; H, 3.11; Cl, 6.44; N, 7.63; S, 5.82. Found: C, 56.64; H, 3.05; Cl, 6.40; N, 7.60; S, 5.78.

5-Chloro-N-(4-(4-(2-chlorophenyl)-5-cyano-1,6-dihydro-6-thioxopyridin-2-yl)phenyl)-2-methoxybenzamide (5c):

Yield 95%, mp. 196-198°C; IR (KBr, cm^{-1}) ν: 3528 (NH), 3419 (SH), 2253 (CN), 1673 (amide I), 1633 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 3.45 (s, 3H, OCH_3), 4.11 (s, 1H, SH), 7.16 (s, 1H, pyrid-H), 7.16-8.06 (m, 11H, Ar-H), 10.98 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 56.72 (OCH_3), 118.40 (CN), 165.22 (CONH), 163.53, 138.66, 145.47, 112.56, 171.36 (5C, pyrid-C), 123.05, 134.26, 127.52, 126.68, 133.88, 128.40, 115.63, 153.30, 139.75, 117.94, 129.80, 144.88, 145.32, 129.76, 131.60, 123.65 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 506 [M^+]. Elemental analysis for $C_{26}H_{17}Cl_2N_3O_2S$ (506.40): Calcd. C, 61.67; H, 3.38; Cl, 14.00; N, 8.30; S, 6.33. Found: C, 61.60; H, 3.32; Cl, 13.96; N, 8.24; S, 6.30.

2.1d Synthesis of ethyl 6-(4-(5-chloro-2-methoxybenzamido) phenyl)-3-amino-4-(aryl)thieno[2,3-b]pyridine-2-carboxylate 6a-c

A solution of the appropriate **5a-c** (1 mmol), ethyl chloroacetate (1 mmol) and sodium ethoxide (68 mg, 10 mmol) in ethanol (10 ml) was refluxed for 4 hrs. The reaction mixture was evaporated under reduced pressure, the obtained residue was solidified with water. The formed solid was filtered off, dried and crystallized from ethyl acetate/methanol to give the corresponding thienopyridine derivatives **6a-c**, respectively.

Ethyl 6-(4-(5-chloro-2-methoxybenzamido) phenyl)-3-amino-4-phenylthieno[2,3-b]pyridine-2-carboxylate (6a):

Yield 70%, mp. 301-303°C; IR (KBr, cm^{-1}) ν: 3581-3496 (NH, NH_2), 1738 (CO), 1673 (amide I), 1628 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 1.31

(t, 3H, CH_3), 3.48 (s, 3H, OCH_3), 4.51 (q, 2H, CH_2), 5.75 (s, 2H, NH_2 exchangeable with D_2O), 7.16 (s, 1H, pyrid-H), 7.24-7.95 (m, 12H, Ar-H), 11.00 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 14.32 (CH_3), 56.70 (OCH_3), 58.32 (OCH_2), 158.70 (CO, ester), 163.75 (CONH), 130.32, 146.85, 127.36, 147.76, 138.98, 160.88, 173.65 (thienopyridyl-C), 134.76, 128.05, 133.52, 128.32, 113.08, 156.90, 141.74, 117.48, 131.12, 142.15, 141.64, 127.74, 128.70, 130.75 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 558 [M^+]. Elemental analysis for $C_{30}H_{24}ClN_3O_4S$ (558.05): Calcd. C, 64.57; H, 4.33; Cl, 6.35; N, 7.53; S, 5.75. Found: C, 64.52; H, 4.28; Cl, 6.30; N, 7.50; S, 5.70.

Ethyl 6-(4-(5-chloro-2-methoxybenzamido) phenyl)-3-amino-4-(4-bromophenyl)thieno[2,3-b]pyridine-2-carboxylate (6b):

Yield 84%, mp. 216-218^{o}C; IR (KBr, cm^{-1}) ν: 3566-3490 (NH, NH_2), 1742 (CO), 1670 (amide I), 1622 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 1.28 (t, 3H, CH_3), 3.52 (s, 3H, OCH_3), 4.54 (q, 2H, CH_2), 5.72 (s, 2H, NH_2 exchangeable with D_2O), 7.10 (s, 1H, pyrid-H), 7.10-8.00 (m, 11H, Ar-H), 10.96 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 14.40 (CH_3), 56.64 (OCH_3), 59.05 (OCH_2), 158.65 (CO, ester), 163.65 (CONH), 130.25, 146.88, 127.42, 147.82, 139.01, 160.81, 173.68 (thienopyridyl-C), 134.12, 127.60, 133.85, 128.42, 115.58, 153.32, 139.80, 118.18, 129.80, 144.56, 145.24, 129.66, 132.12, 123.68 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 637 [M^+]. Elemental analysis for $C_{30}H_{23}BrClN_3O_4S$ (636.94): Calcd. C, 56.57; H, 3.64; Cl, 5.57; N, 6.60; S, 5.03. Found: C, 56.50; H, 3.60; Cl, 5.50; N, 6.54; S, 5.00.

Ethyl 6-(4-(5-chloro-2-methoxybenzamido) phenyl)-3-amino-4-(2-chlorophenyl)thieno[2,3-b]pyridine-2-carboxylate (6c):

Yield 72%, mp. 206-208^{o}C; IR (KBr, cm^{-1}) ν: 3582-3476 (NH, NH_2), 1736 (CO), 1672 (amide I), 1620 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 1.22 (t, 3H, CH_3), 3.48 (s, 3H, OCH_3), 4.50 (q, 2H, CH_2), 5.74 (s, 2H, NH_2 exchangeable with D_2O), 7.14 (s, 1H, pyrid-H), 7.14-8.00 (m, 11H, Ar-H), 10.92 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 14.32 (CH_3), 56.58 (OCH_3), 59.12 (OCH_2), 158.56 (CO, ester), 163.76 (CONH), 130.28, 146.80, 127.40, 147.762, 138.93, 160.76, 173.60 (thienopyridyl-C), 123.00, 134.34, 127.46, 126.56, 133.86, 128.48, 115.66, 153.42, 139.81, 117.98, 129.84, 144.80, 145.24, 129.70, 131.55, 123.60 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 592 [M^+]. Elemental analysis for $C_{30}H_{23}Cl_2N_3O_4S$ (592.49): Calcd. C, 60.81; H, 3.91; Cl, 11.97; N, 7.09; S, 5.41. Found: C, 60.76; H, 3.84; Cl, 11.92; N, 7.00; S, 5.35.

2.1e Synthesis of N-(4-(6-(aryl)-3-cyano-1,2-dihydro-2-oxopyridin-4-yl)phenyl)-5-chloro-2-methoxybenzamide 7a,b

A mixture of chalcone **2a,b** (2 mmol), cyanoacetamide (0.2 g, 2.4 mmol) and sodium methoxide (10 mg, 2 mmol) in methanol (10 ml) was refluxed for 2.5 hrs. The reaction mixture was evaporated under reduced pressure, the obtained solid was washed with water, filtered off, dried and crystallized from methanol to give the corresponding compounds **7a,b**, respectively.

5-Chloro-N-(4-(3-cyano-1,2-dihydro-2-oxo-6-phenylpyridin-4-yl)phenyl)-2methoxybenzamide (7a):

Yield 50%, mp. 147-149^{o}C; IR (KBr, cm^{-1}) ν: 3528 (NH) 2248 (CN), 1748 (CO), 1668 (amide I), 1624 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.39 (s, 3H, OCH_3), 7.11 (s, 1H, pyrid-H), 7.21-7.97 (m, 12H, Ar-H), 8.65 (s, 1H, NH exchangeable with D_2O), 10.48 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.52 (OCH_3), 118.45 (CN), 164.05 (CONH), 158.90, 107.98, 144.76, 131.24, 154.74 (5C, pyrid-C), 135.12, 128.13, 133.42, 127.92, 113.18, 156.88, 141.86, 118.45, 130.82, 141.86, 141.72, 127.66, 128.85, 131.25 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 456 [M^+]. Elemental analysis for $C_{26}H_{18}ClN_3O_3$ (455.89): Calcd. C, 68.50; H, 3.98; Cl, 7.78; N, 9.22. Found: C, 68.43; H, 3.94; Cl, 7.74; N, 9.22.

N-(4-(6-(4-Bromophenyl)-3-cyano-1,2-dihydro-2-oxopyridin-4-yl)phenyl)-5-chloro-2-methoxybenzamide (7b):

Yield 54%, mp. 158-160^{o}C; IR (KBr, cm^{-1}) ν: 3526 (NH), 2248 (CN), 1748 (CO), 1668 (amide I), 1618 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.56 (s, 3H, OCH_3), 7.14 (s, 1H, pyrid-H), 7.16-8.02 (m, 11H, Ar-H), 8.74 (s, 1H, NH exchangeable with D_2O), 10.76 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.66 (OCH_3), 118.32 (CN), 164.98 (CONH), 158.88, 108.08, 144.84, 131.34, 154.82 (5C, pyrid-C), 134.05, 127.60, 133.94, 128.38, 115.52, 153.18, 139.86, 118.11, 129.76, 144.50, 145.18, 129.62, 132.01, 123.68 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 535 [M^+]. Elemental analysis for $C_{26}H_{17}BrClN_3O_3$ (534.79): Calcd. C, 58.39; H, 3.20; Cl, 6.63; N, 7.86. Found: C, 58.33; H, 3.16; Cl, 6.60; N, 7.80.

2.1f Synthesis of N-(4-(6-(aryl)-3-cyano-1,2-dihydro-2-oxopyridin-4-yl)phenyl)-5-chloro-2-methoxybenzamide 8a,b

A mixture of chalcone **2a,b** (2 mmol), thiocyanoacetamide (0.240 g, 2.4 mmol) and sodium ethoxide (136 mg, 2 mmol) in ethanol (10 ml) was refluxed for 4 hrs. The reaction mixture was evaporated under reduced pressure, the obtained solid was washed with water, filtered off, dried and

crystallized from benzene to give the corresponding compounds **8a,b**, respectively.

5-Chloro-N-(4-(3-cyano-1,2-dihydro-6-phenyl-2-thioxopyridin-4-yl)phenyl)-2-methoxybenzamide (8a):

Yield 80%, mp. 236-238°C; IR (KBr, cm^{-1}) ν: 3581 (NH), 3356 (SH), 2248 (CN), 1670 (amide I), 1624 (amide II); ^{1}H-NMR (DMSO-d_6, ppm): δ = 3.41 (s, 3H, OCH_3), 4.38 (s, 1H, SH), 7.16 (s, 1H, pyrid-H), 7.32-8.05 (m, 12H, Ar-H), 11.80 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR (DMSO-d_6, ppm): δ = 56.33 (OCH_3), 118.02 (CN), 163.76 (CONH), 173.05, 116.16, 142.75, 134.92, 154.90 (5C, pyrid-C), 134.73, 127.84, 133.40, 127.84, 112.70, 156.80, 141.60, 117.56, 130.84, 141.88, 141.52, 127.62, 128.62, 130.32 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 472 [M^+]. Elemental analysis for $C_{26}H_{18}ClN_3O_2S$ (471.96): Calcd. C, 66.17; H, 3.84; Cl, 7.51; N, 8.90; S, 6.79. Found: C, 66.13; H, 3.82; Cl, 7.45; N, 8.86; S, 6.73.

N-(4-(6-(4-Bromophenyl)-3-cyano-1,2-dihydro-2-thioxopyridin-4-yl)phenyl)-5-chloro-2-methoxy-benzamide (8b):

Yield 74%, mp. 270-272°C; IR (KBr, cm^{-1}) ν: 3568 (NH), 3346 (SH), 2261 (CN), 1680 (amide I), 1623 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.44 (s, 3H, OCH_3), 3.86 (s, 1H, SH), 7.08 (s, 1H, pyrid-H), 7.24-7.96 (m, 11H, Ar-H), 10.88 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.23 (OCH_3), 117.82 (CN), 163.86 (CONH), 172.56, 116.38, 142.70, 134.84, 154.74 (5C, pyrid-C), 134.12, 127.68, 133.91, 128.42, 115.51, 153.18, 139.89, 118.23, 129.78, 144.56, 145.24, 129.49, 132.15, 123.78 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 551 [M^+]. Elemental analysis for $C_{26}H_{17}BrClN_3O_2S$ (550.85): Calcd. C, 56.69; H, 3.11; Cl, 6.44; N, 7.63; S, 5.82. Found: C, 56.65; H, 3.08; Cl, 6.42; N, 7.61; S, 5.77.

2.1g Synthesis of ethyl 4-(4-(5-chloro-2-methoxybenzamido)phenyl)-3-amino-6-(aryl)thieno[2,3-b]pyridine-2-carboxylate 9a,b

A solution of the appropriate **8a,b** (1 mmol), ethyl chloroacetate (1 mmol) and sodium ethoxide (68 mg, 10 mmol) in absolute ethanol (10 ml) was refluxed for 4 hrs. The reaction mixture was evaporated under reduced pressure, the obtained residue was solidified with water. The formed solid was filtered off, dried and crystallized from ethyl acetate to give the corresponding thienopyridine derivatives **9a,b**, respectively.

Ethyl 4-(4-(5-chloro-2-methoxybenzamido) phenyl)-3-amino-6-phenylthieno[2,3-b]pyridine-2-carboxylate (9a):

Yield 76%, mp. 177-179°C; IR (KBr, cm^{-1}) ν: 3563-3498 (NH, NH_2), 1748 (CO), 1665 (amide I), 1621 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 1.27 (t, 3H, CH_3), 3.38 (s, 3H, OCH_3), 4.48 (q, 2H, CH_2), 5.78 (s, 2H, NH_2, exchangeable with D_2O), 7.09 (s, 1H, pyrid-H), 7.30-7.90 (m, 12H, Ar-H), 11.30 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z (%): 558 [M^+]. Elemental analysis for $C_{30}H_{24}ClN_3O_4S$ (558.05): Calcd. C, 64.57; H, 4.33; Cl, 6.35; N, 7.53; S, 5.75. Found: C, 64.53; H, 4.30; Cl, 6.30; N, 7.50; S, 5.70.

Ethyl 4-(4-(5-chloro-2-methoxybenzamido) phenyl)-3-amino-6-(4-bromophenyl)thieno[2,3-b]pyridine-2-carboxylate (9b):

Yield 68%, mp. 134-136°C; IR (KBr, cm^{-1}) ν: 3560-3489 (NH, NH_2), 1745 (CO), 1665 (amide I), 1620 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 1.31 (t, 3H, CH_3), 3.42 (s, 3H, OCH_3), 4.49 (q, 2H, CH_2), 5.75 (s, 2H, NH_2, exchangeable with D_2O), 7.12 (s, 1H, pyrid-H), 7.18-8.05 (m, 11H, Ar-H), 11.00 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 14.48 (CH_3), 56.17 (OCH_3), 59.65 (CH_2), 158.75 (CO, ester), 163.76 (CONH), 130.34, 143.70, 127.10, 134.70, 155.10, 173.81, 146.76 (7C, thienopyrid-C), 134.65, 127.90, 134.68, 125.83, 113.51, 154.80, 141.27, 117.15, 131.71, 141.80, 124.10, 131.90, 130.70, 142.69 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 637 [M^+]. Elemental analysis for $C_{30}H_{23}BrClN_3O_4S$ (636.94): Calcd. C, 56.57; H, 3.64; Cl, 5.57; N, 6.60; S, 5.03. Found: C, 56.52; H, 3.60; Cl, 5.51; N, 6.55; S, 5.00.

2.1h Synthesis of N-(4-(6-amino-4-(aryl)-5-cyanopyridin-2-yl)phenyl)-5-chloro-2-methoxy-benzamide 10a,b

Method A:

A mixture of **2a,b** (2 mmol), malononitril (0.16 g, 2.4 mmol) and ammonium acetate (1.24 g, 16 mmol) in n-butanol (25 ml) was refluxed for 3 hrs. After cooling, the precipitated solid product was filtered off, washed with water, dried and crystallized from acetone/methanol to give the corresponding cyanoaminopyridine derivatives **10a,b**, respectively.

Method B:

A solution of **3** (2 mmol), malononitril (0.16 g, 2.4 mmol), aromatic aldehydes, namely, benzaldehyde or p-bromobenzaldehyde (2 mmol) and ammonium acetate (1.24 g, 16 mmol) in n-butanol (20 ml) was refluxed for 2 hrs. After cooling, the obtained precipitate was collected by filtration, washed with water, dried and crystallized from from acetone/methanol to give the corresponding cyanoaminopyridine derivatives **10a,b**, respectively.

N-(4-(6-Amino-5-cyano-4-phenylpyridin-2-yl)phenyl)-5-chloro-2-methoxybenzamide (10a):

Yield 82% [A], 90% [B], mp. 211-213°C; IR (KBr, cm^{-1}) ν: 3548-3495 (NH, NH_2), 2231 (CN), 1671 (amide I), 1628 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.47 (s, 3H, OCH_3), 5.44 (s, 2H, NH_2 exchangeable with D_2O), 6.96 (s, 1H, pyrid-H), 7.08-7.92 (m, 12H,

Ar-H), 11.32 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.18 (OCH_3), 116.29 (CN), 164.75 (CONH), 163.90, 138.42, 145.14, 111.50, 176.11 (5C, pyrid-C), 134.76, 127.77, 133.30, 127.83, 112.66, 156.55, 141.47, 117.70, 130.80, 141.32, 141.43, 127.78, 128.78, 130.20 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 455 [M^+]. Elemental analysis for $C_{26}H_{19}ClN_4O_2$ (455.91): Calcd. C, 68.65; H, 4.21; Cl, 7.79; N, 12.32. Found: C, 68.60; H, 4.16; Cl, 7.73; N, 12.28.

N-(4-(6-Amino-4-(4-bromophenyl)-5-cyanopyridin-2-yl)phenyl)-5-chloro-2-methoxybenzamide (10b): Yield 72% [A], 7%4 [B], mp. 228-230^{o}C; IR (KBr, cm^{-1}) ν: 3556-3490 (NH, NH_2), 2231 (CN), 1668 (amide I), 1622 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.48 (s, 3H, OCH_3), 5.52 (s, 2H, NH_2 exchangeable with D_2O), 6.98 (s, 1H, pyrid-H), 7.12-8.05 (m, 11H, Ar-H), 10.80 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 56.17 (OCH_3), 116.10 (CN), 163.75 (CONH), 163.68, 147.95, 111.50, 171.43, 137.61 (5C, pyrid-C), 134.90, 127.55, 134.23, 124.30, 116.18, 153.15, 140.80, 117.88, 129.12, 141.70, 148.91, 130.70, 131.70, 123.70 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 534 [M^+]. Elemental analysis for $C_{26}H_{18}BrClN_4O_2$ (533.80): Calcd. C, 58.50; H, 3.40; Cl, 6.64; N, 10.50. Found: C, 58.44; H, 3.32; Cl, 6.60; N, 10.45.

2.1k Synthesis of N-(4-(6-(aryl)-3-cyano-2-methoxypyridin-4-yl)phenyl)-5-chloro-2-methoxybenzamide 11a,b

A mixture of **2a,b** (2 mmol), malononitril (0.16 g, 2.4 mmol) and sodium methoxide (0.108 g, 2 mmol) in methanol (25 ml) was refluxed for 3 hrs. The reaction mixture was evaporated under reduced pressure, washed with water, dried and crystallized from methanol to give the corresponding compounds **11a,b**, respectively.

5-Chloro-N-(4-(3-cyano-2-methoxy-6-phenylpyridin-4-yl)phenyl)-2-methoxybenzamide (11a):

Yield 98%, mp. 288-290^{o}C; IR (KBr, cm^{-1}) ν: 3510 (NH), 2236 (CN), 1667 (amide I), 1624 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 3.42 (s, 3H, OCH_3), 4.15 (s, 3H, OCH_3), 7.15 (s, 1H, pyrid-H), 7.23-8.15 (m, 12H, Ar-H), 11.78 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 14.28 (CH_3), 56.19 (OCH_3), 64.21 (OCH_3), 119.21 (CN), 163.27 (CONH), 156.10, 114.70, 147.01, 136.10, 155.80 (5C, pyrid-C), 134.09, 127.21, 133.77, 125.91, 114.97, 155.18, 137.12, 117.60, 131.90, 1456.10, 140.70, 127.83, 128.71, 130.26 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 470 [M^+]. Elemental analysis for $C_{27}H_{20}ClN_3O_3$ (469.92): Calcd. C, 69.01; H, 4.29; Cl, 7.54; N, 8.94. Found: C, 68.92; H, 4.24; Cl, 7.50; N, 8.90.

N-(4-(6-(4-Bromophenyl)-3-cyano-2-methoxypyridin-4-yl)phenyl)-5-chloro-2-methoxybenzamide (11b):

Yield 78%, mp. 298-300^{o}C; IR (KBr, cm^{-1}) ν: 3491 (NH), 2228 (CN), 1671 (amide I), 1621 (amide II); MS (EI, 70 eV): m/z (%): 549 [M^+]. Elemental analysis for $C_{27}H_{19}BrClN_3O_3$ (548.82): Calcd. C, 59.09; H, 3.49; Cl, 6.46; N, 7.66. Found: C, 59.00; H, 3.45; Cl, 6.40; N, 7.60.

2.1l Synthesis of N-(4-(6-(aryl)-3-cyano-2-ethoxypyridin-4-yl)phenyl)-5-chloro-2-methoxy-benzamide 12a,b

A mixture of **2a,b** (2 mmol), malononitril (0.16 g, 2.4 mmol) and sodium ethoxide (0.108 g, 2 mmol) in ethanol (25 ml) was refluxed for 3 hrs. The reaction mixture was evaporated under reduced pressure, washed with water, dried and crystallized from methanol to give the corresponding compounds **12a,b**, respectively.

5-Chloro-N-(4-(3-cyano-2-ethoxy-6-phenylpyridin-4-yl)phenyl)-2-methoxybenzamide (12a):

Yield 86%, mp. 213-215^{o}C; IR (KBr, cm^{-1}) ν: 3512 (NH), 2248 (CN), 1681 (amide I), 1622 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 1.75 (CH_3), 3.41 (t, 3H, OCH_3), 5.07 (q, 2H, OCH_2), 7.17 (s, 1H, pyrid-H), 7.25-8.08 (m, 12H, Ar-H), 11.94 (s, 1H, NH exchangeable with D_2O); ^{13}C-NMR ($CDCl_3$, ppm): δ = 14.28 (CH_3), 54.98 (OCH_3), 57.11 (OCH_2), 116.71 (CN), 163.75 (CONH), 156.91, 114.90, 147.77, 135.50, 154.75 (5C, pyrid-C), 133.90, 127.11, 133.78, 125.97, 114.97, 155.32, 136.81, 117.88, 130.54, 146.70, 141.50, 127.70, 128.90, 130.25 (18C, Ar-C); MS (EI, 70 eV): m/z (%): 484 [M^+]. Elemental analysis for $C_{28}H_{22}ClN_3O_3$ (483.95): Calcd. C, 69.49; H, 4.58; Cl, 7.33; N, 8.68. Found: C, 69.44; H, 4.53; Cl, 7.30; N, 8.62.

N-(4-(6-(4-Bromophenyl)-3-cyano-2-ethoxypyridin-4-yl)phenyl)-5-chloro-2-methoxybenzamide (12b):

Yield 82%, mp. 235-237^{o}C; IR (KBr, cm^{-1}) ν: 3512 (NH), 2248 (CN), 1672 (amide I), 1624 (amide II); ^{1}H-NMR ($CDCl_3$, ppm): δ = 1.65 (t, 3H, CH_3), 3.45 (s, 3H, OCH_3), 5.10 (q, 2H, CH_2), 7.12 (s, 1H, pyrid-H), 7.41-8.23 (m, 11H, Ar-H), 11.23 (s, 1H, NH exchangeable with D_2O); MS (EI, 70 eV): m/z (%): 563 [M^+]. Elemental analysis for $C_{26}H_{21}BrClN_3O_3$ (562.84): Calcd. C, 59.75; H, 3.76; Cl, 6.30; N, 7.47. Found: C, 59.70; H, 3.71; Cl, 6.24; N, 7.42.

2.2. Pharmacological Screening

2.2a Determination of acute toxicity (LD_{50})

The LD_{50} for compounds were determined by injected different gradual increased doses of the tested compounds to adult mail albino rats, then calculate the dose cause 50% animal death, according to Austen and Brocklehurst, (1961).

4.2b Anti-inflammatory activity

Carrageenan® induced rat's paw

Procedure

Groups of adult male albino rats (150-180 g), each of 8 animals were orally dosed with tested compounds at a dose level of 25-50 mg/kg one hour before Carrageenan® challenge. Foot paw edema was induced by subplenter injection of 0.05 ml of 1% suspension of Carrageenan® in saline into the planter tissue of one hind paw. An equal volume of saline was injected to the other hind paw and served as control. Four hours after drug administration the animals were decapitated, blood was collected and the paws were rapidly excised.

The average weight of edema was examined for the treated as well as the control group and the percentage inhibition of weight of edema was also evaluated. Prednisolone® (5 mg/kg) was employed as standard reference against which the tested compounds were compared.

Calculation and evaluation

Thirty minutes after the rats are challenged by subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the planter side of the lift hind paw. The paw is marked with ink at the level of the lateral malleolus; the paw volume was measured by a sensitive method developed by **Webb and Griswold, (1984)** that calculated by interfacing a yridi DeltaRange top-loading balance with a micro computer.

% Protection = (A – B) X 100 / A

A = the paw volume of non-treated group

B = the paw volume of treated group

Estimation of plasma prostaglandin E2 (PGE2)

Procedure

Heparinized blood samples were collected from rats obtained from the previous pyridines zation examined groups (n = 8), plasma was separated by centrifugation at 12000 g for 2 min at 40^{o}C and immediately stored frozed -2^{o}C until use.

The design correlate EIA prostaglandin E2 (PGE2) kit (Merck, Darmstadt, Germany) is a competitive pyridi assay for the quantitative determination of PGE2 in biological fluids. The kit uses a monoclonal antibody to PGE2 to bind, in a competitive manner, the PGE2 in the sample after a simultaneous incubation at room temperature. The excess reagents were washed away and the substrate was added, after a short incubation time the enzyme reaction was stopped and the yellow colour generated was read on a micro plate reader (DYNATCh, MR 5000) at 405 nm. The intensity of the bound yellow colour is inversely proportional to the concentration of PGE2 in either standard or samples.

Calculation and evaluation

The PGE2 was calculated for the treated and control groups, then the PGE2 percentage inhibition is determined by the following equation:

% inhibition = (A – B) X 100 / A

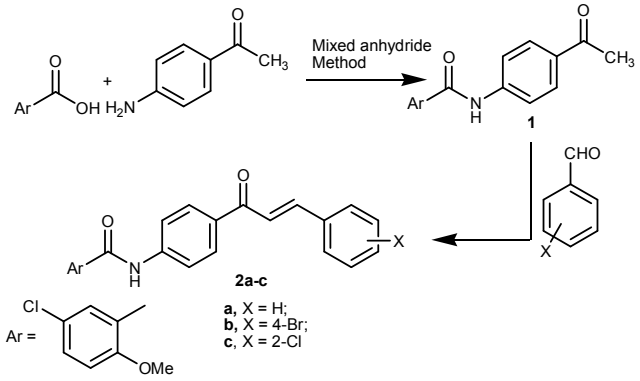
A = PGE2 in the control group

B = PGE2 in the treated group

3. Results and discussion

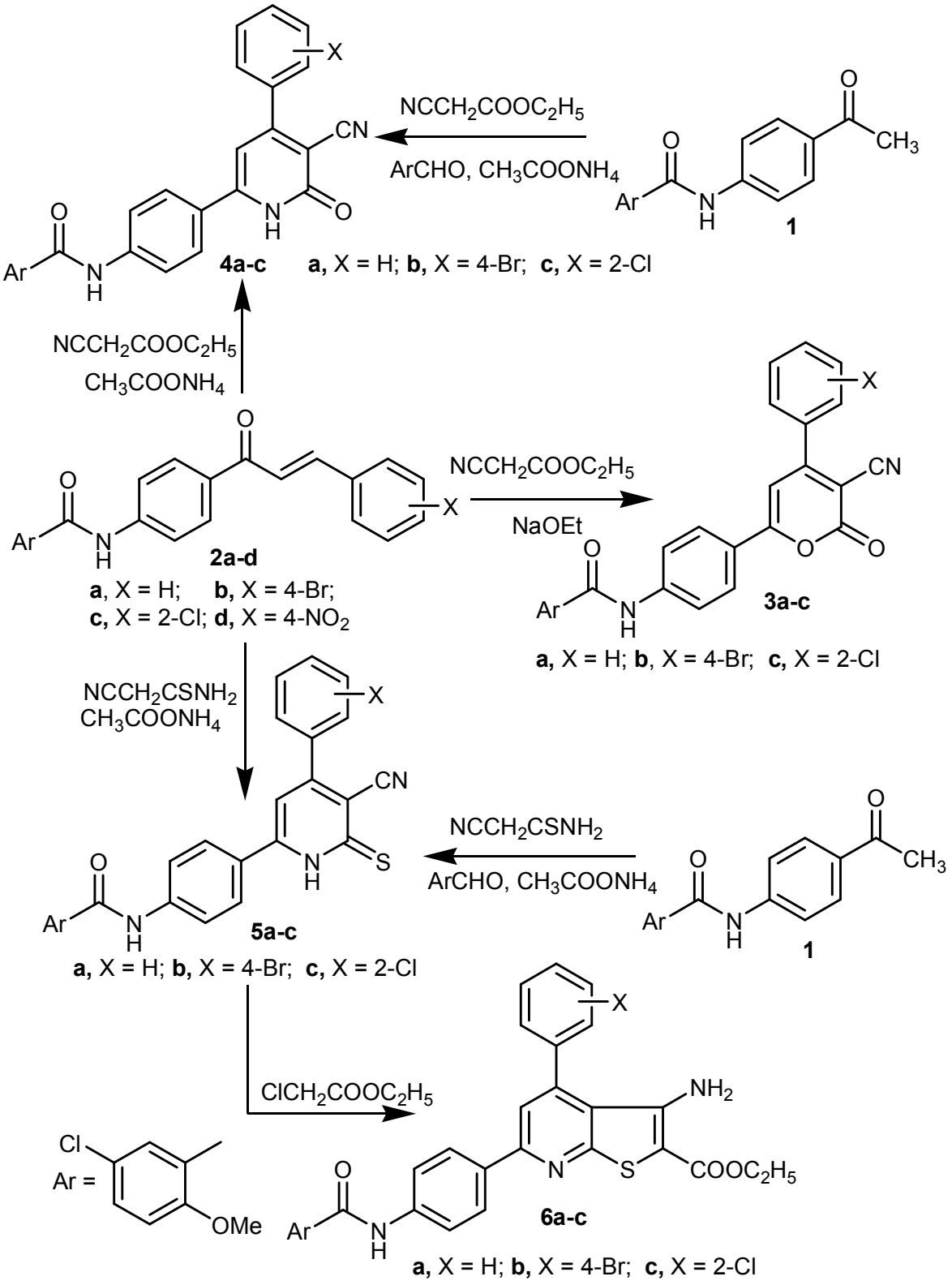
3.1. Synthesis

The continuation of our previous work, a series of substituted pyridine derivatives were synthesized according to our previous reported procedures [Abdulla et al., 2013a; Abdulla et al., 2013b] using N1-[4-(substituted acryloyl)phenyl]-5-chloro-2-methoxybenzamide derivatives **2a-c** as starting materials (Scheme 1).

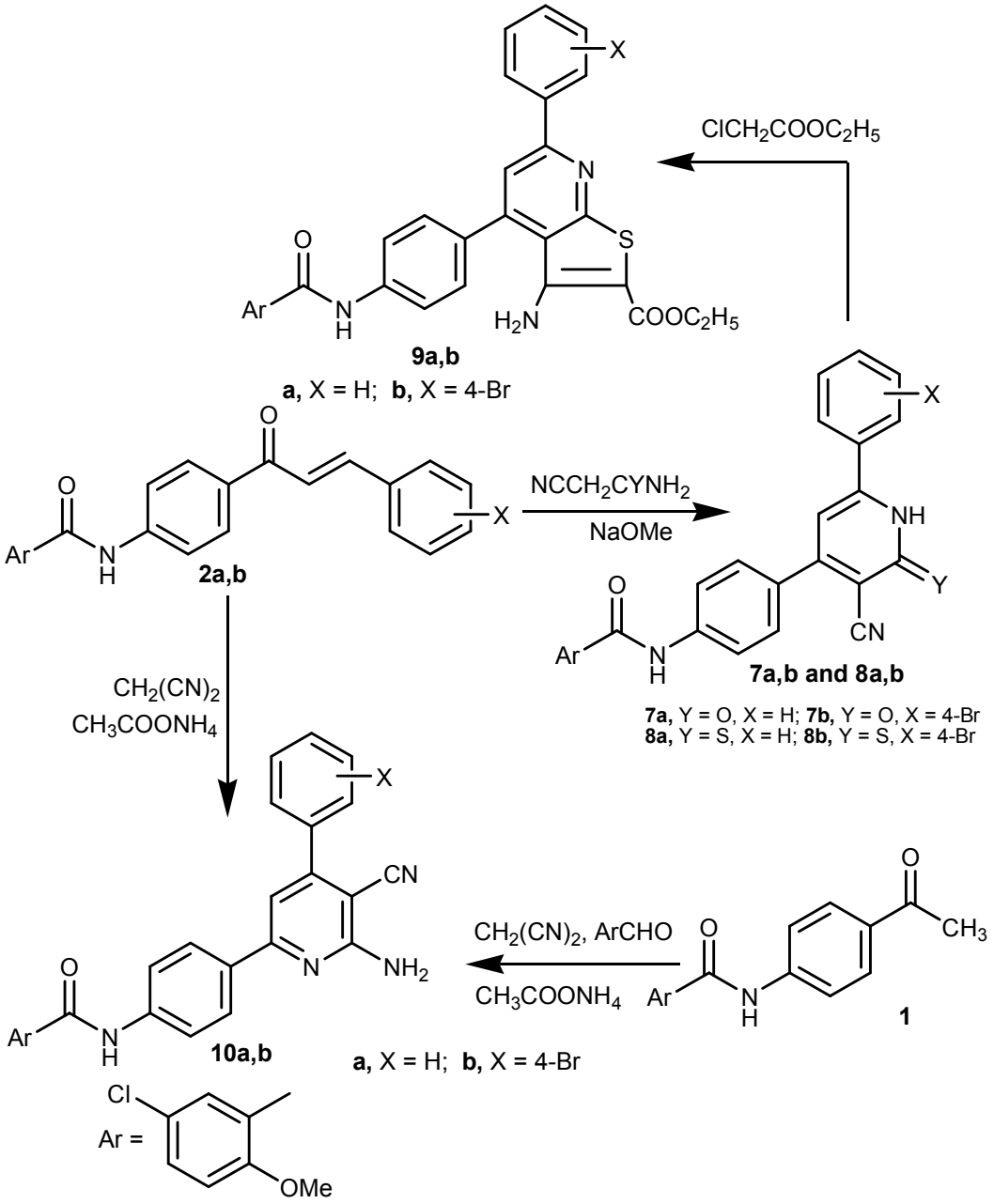


Scheme 1. Synthetic routes of starting materials **2a-c**

Cyclocondensation of **2a-c** with ethyl cyanoacetate in the presence of sodium ethoxide gave the corresponding 2-oxopyranoyl derivatives **3a-c**, respectively, but, when **2a-c** condensed with ethyl cyanoacetate in the presence of ammonium acetate gave the corresponding cyanopyridone derivatives **4a-c**, which was prepared directly from acetyl derivative **1** with ethyl cyanoacetate and appropriate aromatic aldehyde in the presence of ammonium acetate. Similarly, condensation of **2a-c** with ethyl thiocyanoacetamide in the presence of ammonium acetate gave the corresponding 2-thioxopyridinyl derivatives **5a-c**, respectively, which was prepared directly from acetyl derivative **1** with thiocyanoacetamide and appropriate aromatic aldehyde in the presence of ammonium acetate. In additionally, treatment of **5a-c** with ethyl chloroacetate in the presence of sodium ethoxide afforded the corresponding thinopyridine derivatives **6a-c**, respectively (Scheme 2).



Scheme 2. Synthetic routes of compounds **3-6**

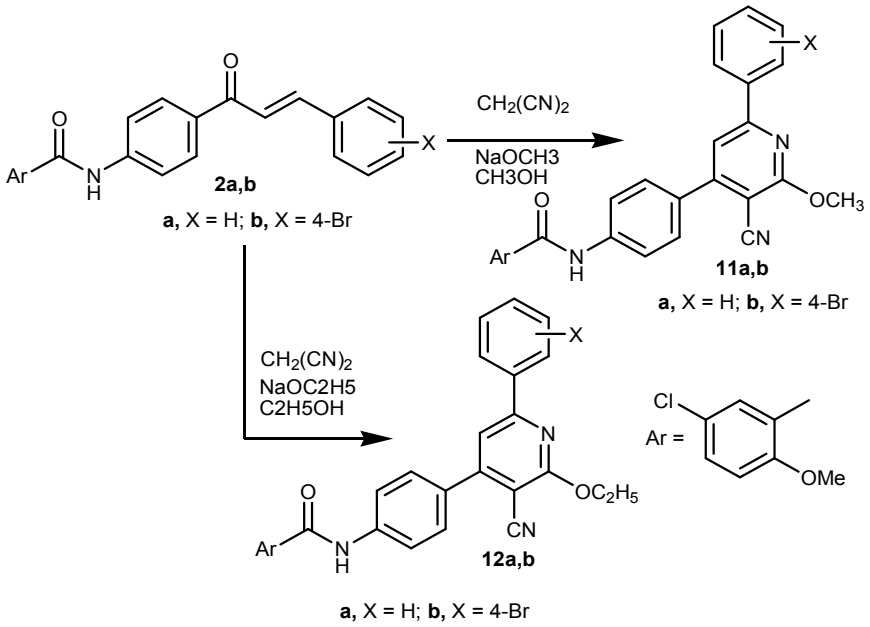


Scheme 3. Synthetic routes of compounds **7-10**

Finally, compounds **2a,b** was treated with malononitril in methanolic sodium methoxide afforded the corresponding cyanomethoxy pyridine derivatives **11a,b**. When the latter reaction completed in ethanolic sodium ethoxide afforded the corresponding cyanoethoxy pyridine derivatives **12a,b**, respectively (Scheme 4).

3.2. Pharmacological screening

All animals were obtained from the Animal House Colony, Research Institute of Ophthalmology, Giza, Egypt. The ethical committee of the National Research Centre, Cairo, Egypt, approved the protocol of this study. Initially the acute toxicity of the compounds was assayed via the determination of their LD_{50} (Table 1). All the compounds except **2c, 3b, 4c, 7b, 8b** and **10a** were interestingly less toxic than the reference drug (Table 1). The newly synthesized compounds were then pharmacologically screened on male albino rats for their anti-inflammatory potency (Tables 2 and 3). Regarding the protection against carrageenan-induced edema, some of the tested compounds, were found to be more potent than Prednisolone®.



Scheme 4. Synthetic routes of compounds **11** and **12**

Table 1. **Acute toxicity (LD_{50}) of the synthesized compounds**

Compound N°	LD_{50} [mg/kg]
2a	3.718 ± 0.011
2b	1.978 ± 0.014
2c	1.465 ± 0.012
3a	4.176 ± 0.014
3b	1.518 ± 0.012
3c	1.812 ± 0.011
4a	2.480 ± 0.013
4b	3.060 ± 0.011
4c	1.512 ± 0.011
5a	2.580 ± 0.011
5b	2.710 ± 0.013
5c	1.813 ± 0.012
6a	3.610 ± 0.011
6b	2.816 ± 0.012
6c	3.614 ± 0.012
7a	1.918 ± 0.014
7b	1.523 ± 0.012
8a	2.547 ± 0.016
8b	1.308 ± 0.012
9a	1.739 ± 0.011
9b	2.313 ± 0.013
10a	1.210 ± 0.012
10b	2.012 ± 0.013
11a	2.122 ± 0.013
11b	2.020 ± 0.010
12b	2.810 ± 0.016
12a	3.110 ± 0.015
Prednisolone®	1.618 ± 0.016

2.2a Purpose and rational

For the determination of the antiphlogistic potency of the synthesized compounds, two standard tests were realized at 25 and 50 mg/kg rat body weight namely, the protection against Carrageenan® induced edema according Winter *et* al. (1962) and the inhibition of plasma PGE2. The later is known as a good confirming indicator for the Carrageenan® induced rat paw edema [Herrmann et al., 1990].

2.2b Anti-inflammatory screening

Regarding the protection against Carrageenan® induced edema, eight compounds namely **2a, 2b, 3a, 4a, 5a, 5b, 6a, 6b, 6c, 8a, 9b, 12a** and **12b** were found more potent than Prednisolone®. Where, their protection percentage against carrageenan induced edema at two dose levels 25 and 50 mg/kg are 94.66/95.72, 93.11/94.13, 93.60/99.18, 88.18/99.45, 93.65/95.14, 88.26/98.22, 92.17/99.20, 88.22/99.46, 92.15/99.19, 88.14/98.17, 93.41/96.16, 93.67/95.13 and 93.15/94.12, respectively (Prednisolone® 81/93). On the other hand, the inhibition of plasma PGE2 for the compounds **2b, 3a, 4a, 6a, 6b, 9b, 12a** and **12b** were found more potent than Prednisolone® at two tested doses levels 25 and 50 mg/kg. The inhibition percentage for the latter compounds was found as: 90.40/93.20, 93.35/96.56, 86.26/91.62, 85.62/95.18, 85.26/91.62, 89.34/93.25, 92.33/96.50 and 85.62/95.10, respectively.

Table 2. Anti-inflammatory potencies of the synthesized compounds (protection against carrageenan-induced edema).

Compound N°	Dose [mg/kg]	Protection against carrageenan-induced edema [%]*
2a	25 50	94.66 ± 0.069 95.72 ± 0.070
2b	25 50	93.11 ± 0.068 94.13 ± 0.079
2c	25 50	58.26 ± 0.072 79.16 ± 0.064
3a	25 50	93.60 ± 0.089 99.18 ± 0.086
3b	25 50	55.22 ± 0.053 66.17 ± 0.063
4a	25 50	88.18 ± 0.060 99.45 ± 0.076
4b	25 50	53.34 ± 0.080 56.34 ± 0.076
4c	25 50	91.17 ± 0.092 92.88 ± 0.082
5a	25 50	93.65 ± 0.081 95.14 ± 0.076
5b	25 50	88.26 ± 0.077 98.22 ± 0.078
5c	25 50	52.34 ± 0.083 58.24 ± 0.078
6a	25 50	92.17 ± 0.079 99.20 ± 0.074
6b	25 50	88.22 ± 0.060 99.46 ± 0.078
6c	25 50	92.15 ± 0.075 99.19 ± 0.078
7a	25 50	63.89 ± 0.064 84.20 ± 0.065
7b	25 50	56.74 ± 0.068 75.16 ± 0.074
8a	25 50	88.14 ± 0.076 98.17 ± 0.077
8b	25 50	55.74 ± 0.069 75.16 ± 0.074
9a	25 50	65.80 ± 0.075 88.44 ± 0.081
9b	25 50	93.41 ± 0.087 96.16 ± 0.084
10a	25 50	44.17 ± 0.055 72.13 ± 0.066
10b	25 50	54.22 ± 0.067 73.17 ± 0.045
11a	25 50	53.16 ± 0.078 65.18 ± 0.065
11b	25 50	47.18 ± 0.080 63.17 ± 0.054
12a	25 50	93.67 ± 0.079 95.13 ± 0.076
12b	25 50	93.15 ± 0.066 94.12 ± 0.077
Prednisolone®	25 50	81.00 ± 0.100 93.00 ± 0.082

* The doses tested were 25, 50 mg and carryout three determinations for each dose.

Table 3. Anti-inflammatory potencies of the synthesized compounds (Inhibition of plasma PGE2).

Compound N°	Dose [mg/kg]	Inhibition of plasma PGE2 [%]*
2a	25 50	48.26 ± 0.080 79.75 ± 0.081
2b	25 50	90.40 ± 0.086 93.20 ± 0.095
2c	25 50	78.54 ± 0.095 82.62 ± 0.086
3a	25 50	93.35 ± 0.086 96.56 ± 0.111
3b	25 50	72.15 ±0.121 73.56 ± 0.101
4a	25 50	86.26 ± 0.089 91.62 ± 0.101
4b	25 50	44.28 ± 0.080 63.13 ± 0.078
4c	25 50	78.62 ± 0.093 82.65 ± 0.083
5a	25 50	82.32 ± 0.077 79.25 ± 0.072
5b	25 50	46.33 ± 0.091 61.36 ± 0.110
5c	25 50	38.42 ± 0.110 69.26 ± 0.094
6a	25 50	85.62 ± 0.112 95.18 ± 0.122
6b	25 50	85.26 ± 0.088 91.62 ± 0.100
6c	25 50	47.62 ± 0.064 71.55 ± 0.086
7a	25 50	43.18 ± 0.086 63.13 ± 0.078
7b	25 50	41.16 ± 0.077 54.17 ± 0.092
8a	25 50	52.16 ± 0.084 76.18 ± 0.088
8b	25 50	50.99 ± 0.101 72.00 ± 0.098
9a	25 50	46.31 ± 0.088 62.38 ± 0.110
9b	25 50	89.34 ± 0.085 93.25 ± 0.094
10a	25 50	76.55 ± 0.078 85.87 ± 0.081
10b	25 50	53.11 ± 0.088 74.82 ± 0.079
11a	25 50	76.42 ± 0.086 81.58 ± 0.083
11b	25 50	53.10 ± 0.080 73.80 ± 0.076
12a	25 50	92.33 ± 0.087 96.50 ± 0.110
12b	25 50	84.64 ± 0.110 95.10 ± 0.120
Prednisolone®	25 50	77.0 ± 0.084 91.0 ± 0.087

* The doses tested were 25, 50 mg and carryout three determinations for each dose.

4. Conclusion

We have synthesized and tested two series of pyridin-2-yl)phenyl)-2-methoxybenzamide and thieno[2,3-b]pyridine derivatives for their anti-inflammatory activities. Twenty six compounds namely induced significant activity. The un-substituted phenyl derivatives showed better results than the halogenated derivatives. All the compounds except **2d, 2f, 3b, 4c, 7b, 8b** and **10a** were interestingly less toxic than the reference drug (Table 1). The newly synthesized compounds were then pharmacologically screened on male albino rats for their anti-inflammatory potency (Tables 2 and 3). Regarding the protection against carrageenan-induced edema, some of the tested compounds, were found to be more potent than Prednisolone®.

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