Synthesis and Biological evaluation of pyrrolo[2, 3-*d*]pyrimidine derivatives as antibacterial and antiviral

Khaled M. Hassan Hilmy[*1], Mounir D. Elsafty[2], Alaa Ramadan. I. Morsy[2], Ghada M. Elsadek Aly[2] and Salah M. El-kousy[1]

[1] Chemistry Department, Faculty of Science, El-Menofia University, Shebin El-Kom, Egypt.
[2] Central Laboratory for Evaluation of Veterinary Biologics, Agricultural Research Center, Abbasia, Cairo, Egypt.
hilmykhaled@yahoo.com khaledhilmy@hotmail.com

Abstract: A new class of pyrrolo[2,3-*d*] pyrimidine derivatives has been designed and synthesized, then examined and evaluated for their antiviral and antibacterial activity. New prophylactic therapeutic tools are needed for the treatment of highly pathogenic avian influenza (HPAI) H5N1 and highly virulent Newcastle disease virus (NDV). **3a, 3b, 3g, 3h** have shown to posses highly potent against highly pathogenic avian influenza (HPAI) H5N1 virus and **1a, 2a** are shown highly potent against highly virulent Newcastle disease virus (NDV),and the compounds **1a-c , 2a, 2b, 3a, 3g, 3h** are shown highly potent against enterobacteriases [*Escherichia coli* and *Salmonella typhimurium*] strains. This study is the first record in Egypt and may be in the world concerning the activity of these new class of pyrrolo[2,3-*d*]pyrimidine derivatives against (HPAI) H5N1 and NDV.
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1. Introduction:

Compounds containing pyrrolopyrimidine functional groups, collectively referred to as 7-deazapurines, are a structurally diverse class of nucleoside analogs with demonstrated antibiotic [1]. These compounds have been intensively investigated as antitumor [2,3], Cytotoxicity[4] anti-allergic[5], antiviral[6], antimicrobial and anti-inflammatory agents [7].

During the past ten years a series of avian epidemics, significant reemergence of highly pathogenic avian influenza (HPAI H5N1) has been reported in several Asian, countries including Hong Kong, Korea, Japan, Taipei, Cambodia, China, Indonesia and Thailand, with additionally confirmed cases of human death in Vietnam [8].

With continued outbreaks of the H5N1 virus in poultry and wild birds, further human cases are likely, and the potential for the emergence of a human adapted H5N1 virus either by reassortment or mutation, is a threat of public health worldwide. Limitation in our current HPAI treatment options and the continuing out breaks of the H5N1 viruses have contributed to a growing need for new and effective chemotherapeutic agents to treat viral diseases.

Also New Castle disease is a highly contagious, septicaemic, fatal and destructive disease which attack chiefly chickens and turkeys usually in an acute, sometimes in sub acute or even chronic form. Occasionally human being and even wild birds may be also infected with the virus. New castle disease (ND) caused by virulent virus, is one of the most infectious diseases of poultry and induces up to 100% mortality as well as a decrease in egg production and poor egg quality [9]. ND is regarded throughout the world as one of the most important diseases of poultry, not only due to the serious disease and high flock mortality that may result from some ND virus (NDV) infections, but also through the economic impact that may ensue due to trading restrictions and embargoes placed on areas and countries where outbreaks have occurred [10].

On the bases of these observations, we report the synthesis of a novel series of pyrrolo[2,3-*d*] pyrimidine derivatives and evaluate them as antiviral and antibacterial.

2. Materials and Methods

Biological activity

Pyrroles **1a-c** and Pyrrolo[2,3-*d*]pyrimidine derivatives **2a-c, 3a-i** synthesized as described before, stock solutions of all compounds were prepared at 10 mg /ml in dimethyl sulfoxide then make different concentration.

Highly pathogenic avian influenza (HPAI) H5N1 virus A/chicken/Egypt/9402 NAMRU3-CLEVB 213/2007 (H5N1) of accession No.EU 623467 obtained from Central laboratory for Evaluation of Veterinary Biologics (CLEVB), was used at titer of 106 EID 50/ml (Embryo infective dose fifty per ml).

New Castel Disease Virus (NDV) local virulent strain obtained from viral strain bank of Central Laboratory for Evaluation of Veterinary Biologics (CLEVB), NDV was propagated by using SPF chicken eggs, via allantoic sac route and stored at -70°C until used its titer was 10^6 of EID_{50} /ml Growth medium, MEM (minimum essential medium) produced by (Gibco), Was supplemented with 10% new porn calf serum at pH about 7.2 for primary and secondary cultures. Trypsin (1.250) produced by (Gibco), used in a concentration of 0.25% as a cell dispersing agent [11]. Cell culture of African green monkey kidney cells(Vero cell)[12].

Specific pathogen free (SPF) embryonated chicken eggs (ECEs) were used at nine days old and inoculated via allantoic sac route.

Escherichia coli and Salmonella typhimurium obtained from bacterial strain bank of Central Laboratory for Evaluation of Veterinary Biologics (CLEVB).

Mueller-Hinton broth (oxide), this medium was used in the propagation of the bacterial culture. Mueller-Hinton agar[13], this medium was used in the disc diffusion test .it produces a large and clear zone of inhibition when sensitive organisms come in contact with the susceptible antimicrobials compounds.

Toxicity using embryonating chicken eggs (ECEs)

Five hundred and twenty five SPF embryonating chicken eggs (ECEs) of 9 days old were used as following:

Each compound have 6 dilution (ten-fold dilution) and in each dilution we use 5 (ECEs) inoculate with 0.2 ml/ECE via allantoic sac and incubate all eggs for 5 days; through each all eggs candled every day.

Cytotoxicity assays

Various concentration (6 dilution of each compounds) were added to confluent CEF cell and

Vero cell monolayer cultures, 24hr after seeding are maintained for 4 days then viable cell counts of each culture were determined daily by inverted microscopic inspection of cells that`s not affected by compounds .

Antiviral activity

Using SPF embryonating chicken eggs (ECEs) as described in exp. (1)

Equal volume of New castle disease Virus (NDV) was mixed with equal volume of non toxic concentration of each compound and incubated for one hour at room temperature then inoculated into allantoic sac of five ECEs for each concentration of each compound at a dose of 0.2 ml/ECE.

Also in each compound there are 5 ECEs were inoculated with the (NDV) that mixed with equal volume of saline at a dose 0.2 ml/ECE (positive control), also another 5 ECEs were inoculated with 0.2 ml/ECEs of saline alone (negative control), then all the ECEs were incubated at 37 ^{0}C and candled every two hours till all positive controls ECEs were died.

All the embryos of the positive controls were died and allantoic fluid of each was positive for haemagglutination assay (HA), while all the embryos of negative controls were not died and allantoic fluid of each was negative for HA.

Haemagglutination activity of the allantoic fluids of inoculated eggs is measured by micro technique of Haemagglutination test[14].

The previous mentioned steps were repeated with HPIA (H5N1)

Antibacterial activity

Finally, compounds **(1a-c), (2a-c)** and **(3a-i)** were screened for antibacterial activity using disc diffusion method[15].

The microorganisms used in this study were Escherichia coli, sallamonella typhimurium (gram-negative bacteria), all compounds of non toxic concentration of were dissolved in DMSO (100 µg/ml) and 25 µl of them were loaded to 6mm paper discs, 100 µl of 10^9 cell /ml suspension of the micro organisms were spread on sterile Muller-Hinton agar plates and the discs were placed on the surface of culture plates, then incubate for twenty four hour to determine zone of inhibition as shown in Table (3).

Antiviral Effect of Compounds **1a-c, 2a-c** on NDV **(Original virus titer EID_{50} /ml = 10^6)**

Compound	R	R_1	(MIC) µgm/ml	NDE	+ HA	Final EID_{50} of virus
1a	H	H	2.6	0/5	0	0
1b	4-Cl	H	3.2	0/5	0	0
1c	4-Br	H	3.4	0/5	0	0
2a	H	H	2.7	0/5	0	0
2b	4-Cl	H	3.2	2/5	2	10^2
2c	4-Br	H	3.6	0/5	0	0
Ribavirin			3	0/5	0	0

MIC: minimum inhibition concentration.
NDE: no. of died eggs,
+HA: positive haemagglutination.
EID_{50}: (Embryo infective dose fifty per ml).

Table 2. Antiviral Effect of Compounds **3a-i** on H5N1 (Original virus titer EID_{50} /ml=10^6)

compound	(MIC) µgm/ml	NDE	+HA	Final EID_{50} of virus
3a	3.8	0/5	0	0
3b	3.6	1/5	1	10
3c	41	0/5	0	0
3d	40	2/5	2	10^2
3g	4.5	0/5	0	0
3h	4.1	0/5	0	0
3i	49	0/5	0	0
Ribavirin	6	1/5	1	10

MIC: minimum inhibition concentration. NDE: no. of died eggs.
+HA: positive haemagglutination. EID_{50}: (Embryo infective dose fifty per ml).

Table 3. Antibacterial Activity of compounds 1a-c, 2a-c, and 3a-i

Zone of inhibition(mm)					
Compound	**R**	**R_1**	**R_2**	**Escherichia coli**	**Salmonella Typhimurium**
1a	H	H		19	18
1b	4-Cl	H		17	15
1c	4-Br	H		18	17
2a	H	H		19	16
2b	4-Cl	H		19	18
2c	4-Br	H		15	14
3a	H	H	$-CH_2CH_2OCH_2CH_2OH$	14	12
3b	H	H	$-CH_2CHOHCH_2OH$	14	12
3c	H	H	$-(CH_2)_8CH_3$	9	8
3d	4-Cl	H	$-CH_2CH_2OCH_2CH_2OH$	8	8
3e	4-Cl	H	$-CH_2CHOHCH_2OH$	9	8
3f	4-Cl	H	$-(CH_2)_8CH_3$	7	7
3g	4-Br	H	$-CH_2CH_2OCH_2CH_2OH$	14	12
3h	4-Br	H	$-CH_2CHOHCH_2OH$	14	13
3i	4-Br	H	$-(CH_2)_8CH_3$	9	10
Sutrim				13	12

Table 4. MIC (µg/ml) Value of Compounds 1a-c, 2a, 2c & 3a, 3b, 3g, 3h on Escherichia Coli and Salmonella Typhimurium

Comp	R	R_1	R_2	Escherichia coli (µg/ml)	Salmonella typhimurium (µg/ml)
1a	H	H		2.6	2.6
1b	4-Cl	H		2.9	2.9
1c	4-Br	H		3.4	3.4
2a	H	H		2.8	2.8
2b	4-Cl	H		3.2	3.2
2c	4-Br	H		3.6	3.6
3a	H	H	$-CH_2CH_2OCH_2CH_2OH$	3.7	3.7
3b	H	H	$-CH_2CHOHCH_2OH$	3.6	3.6
3g	4-Br	H	$-CH_2CH_2OCH_2CH_2OH$	4.5	4.5
3h	4-Br	H	$-CH_2CHOHCH_2OH$	4.4	4.4
Sutrim				< 4	< 4

Experimental

Chemistry

Melting points were measured with a Gallenkamp apparatus (Weiss-Gallenkamp, London, UK) and are uncorrected. The IR spectra were recorded on KBr pellets on a Jasco FT/IR 460 plus (Japan). ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian Gemini 200 MHz in DMSO-d_6 or $CDCl_3$ as solvent, using tetramethyl-silane (TMS) as internal reference standard. The chemical shifts values are expressed in ppm (parts per million). Elemental analyses were performed by a Vario III CHN analyzer (Germany). All compounds were within ± 0.4% of the theoretical values. Mass spectra were run on DI analysis Shimadzu QP-2010 plus mass spectrometer. All spectroscopic data and elemental analysis were made at the Microanalytical Unit of Cairo University. The progress of the reaction and purity of the compounds were monitored by TLC analytical silica gel plates 60 F_{254}. The chemical reagents used in synthesis were purchased from Fluka, Sigma and Alderish.

General procedure for the synthesis of compounds 1a-c

Derivatives of phenacylmalononitrile were reacted with aniline in the presence of ethanol and conc HCl under reflux to give pyrrole derivatives 1a-c [16].

General procedure for the synthesis of compounds 2a-c

A mixture of 1,5-disubstituted-2-amino-3-cyano-1*H*-pyrrole (1a-c) (0.015mole) in formic acid (20 ml) was refluxed for 4 h. Then the reaction mixture was cooled and the separated solid was filtered, washed with ethanol, dried and crystallized from ethanol.

6,7-Diphenyl-3,7-dihydro-pyrrolo[2,3-*d*] pyrimidin-4-one 2a

Yield: 48%; mp: 292-293 ^{o}C; IR ν_{max} [cm^{-1}]: 3108 (NH), 1680 (C=O), 1596, 1484 (C=C, C=N); ^{1}H NMR (DMSO-d_6) δ ppm: 6.83 (s, 1H, $H_{pyrrole}$), 7.23-7.88 (m, 10H, $H_{arom.}$), 8.10 (s, 1H, $H_{pyrimidine}$), 10.1 (s, 1H, NH); ^{13}C NMR δ ppm: 104.31, 124.62, 128.01, 130.53, 135.39, 139.12, 140.11, 143.41, 147.73, 149.35, 157.69 (aromatic carbons) and 176.34 (C=O); Anal. Calcd for $C_{18}H_{13}N_3O$ (287): C, 75.26; H, 4.52; N, 14.63. Found: C, 75.50; H, 4.43; N, 14.67; MS m/z [%]: 287 [M^+] (100).

6-(4-Chlorophenyl)-7-phenyl-3,7-dihydro-pyrrolo [2,3-*d*]pyrimidin-4-one 2b

Yield: 87%; mp: 320-321 ^{o}C; IR ν_{max} [cm^{-1}]: 3427 (NH), 1668 (C=O), 1588, 1484 (C=C, C=N); ^{1}H NMR ($CDCL_3$) δ ppm: 6.88 (s, 1H, $H_{pyrrole}$), 7.23-7.85 (m, 9H, $H_{arom.}$), 8.13 (s, 1H, $H_{pyrimidine}$), 11.7 (s, 1H, NH); Anal. Calcd for $C_{18}H_{12}N_3ClO$ (321): C, 67.29; H, 3.74; N, 13.1. Found: C, 67.30; H, 3.5; N, 13.2; MS m/z [%]: 321 [M^+] (100).

6-(4-Bromophenyl)-7-phenyl-3,7-dihydro-pyrrolo [2,3-*d*]pyrimidin-4-one 2c

Yield: 84%; mp: 340-341 ^{o}C; IR ν_{max} [cm^{-1}]: 3427 (NH), 1668 (C=O), 1588, 1484 (C=C, C=N); ^{1}H NMR ($CDCL_3$) δ ppm: 6.86 (s, 1H, $H_{pyrrole}$), 7.26-7.87 (m, 9H, $H_{arom.}$), 8.13 (s, 1H, $H_{pyrimidine}$), 11.6 (s, 1H, NH, D_2O exchangeable); Anal. Calcd for $C_{18}H_{12}N_3OBr$ (367): C, 58.86; H, 3.27; N, 11.44. Found: C, 59.23; H, 3.5; N, 11.5; MS m/z [%]: 367 [M^+] (100).

General procedure for the synthesis of 3,6-substituent of Pyrrolo[2,3-*d*]pyrimidin-4-ones 3a-i.

Compounds 2a-c (0.006 mol) of each was suspended in dry DMF (30 ml). To this suspension was added sodium hydride (60%, 0.24 g, 0.006 mol). The mixture was stirred at room temperature for 20 minutes and then the alkyl halide (0.006 mol) was added. The reaction mixture was refluxed for 5 hours. Then the mixture cooled to room temperature and the solvent was evaporated under vacuum. The residue was dissolved in dichloromethane (200 ml), and the organic phase was washed with water (2 x 50 ml). The organic layer was dried over sodium sulfate and evaporated. The residue was purified by chromatography on silica gel column (2 x15 cm) and the product was eluted with $C_2H_5COOCH_3$ / $CHCl_3$.

3-{2-(2-Hydroxyethoxy)ethyl}-6,7-diphenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one 3a

Yield (75%); m.p.280-281^{0}C; IR (KBr) ν_{max} [cm^{-1}]: 1678 (CO), 3419(OH); ^{1}H NMR (DMSO) δ ppm: 2.34 (s, 1H, OH), 3.09-3.15 (t, 2H, N-CH_2), 3.48-3.56 (d, 2H, O-CH_2CH_2OH), 3.63-3.71 (d, 2H, N-CH_2CH_2),3.76-3.82 (t, 2H, CH_2OH) 6.83 (s, 1H, $H_{pyrrole}$), 7.23-7.88 (m, 10H, $H_{arom.}$), 8.10 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 374.95 [M^+] (12). Anal.Calcd. for $C_{22}H_{21}N_3O_3$ (375.42): C, 70.38; H, 5.64; N, 11.19.Found: C, 70.45; H, 5.25; N, 11.05.

3-(2,3-Dihydroxypropyl)-6,7-diphenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one 3b

Yield (75%); m.p.283-285^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1686 (CO), 3403(OH); ^{1}H NMR (DMSO) δ ppm: 2.49 (s, 1H, OH), 2.70-2.72 (d, 2H, N-CH_2), 2.87-2.88 (d, 2H, OH-CH_2), 3.25-3.39 (m, 1H, CH), 6.89 (s, 1H, $H_{pyrrole}$), 7.15-7.89(m, 10H, $H_{arom.}$), 8.11 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 361.05[M^+] (7). Anal.Calcd. for $C_{21}H_{19}N_3O_3$ (361.39): C, 69.79; H, 5.30; N, 11.63.Found: C, 70.45; H, 5.25; N, 11.35.

3-Nonyl-6,7-diphenyl-3H-pyrrolo[2,3 *d*]pyrimidin-4(7H)-one 3c

Yield (65%); m.p.125-127^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1684 (CO); ^{1}H NMR (DMSO) δ ppm: 0.71-0.89 (t, 3H, CH_3), 1.20–1.67 (m, 10H, $5CH_2$), 2.45-2.59 (m, 2H, CH_2), 3.19-3.43 (t, 2H, N-CH_2), 6.85 (s, 1H, $H_{pyrrole}$), 7.11-7.57 (m, 10H, $H_{arom.}$), 8.21 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 413 [M$^+$] (100). Anal.Calcd. for $C_{27}H_{31}N_3$ O (413.55): C, 78.42; H, 7.56; N, 10.16.Found: C, 78.75; H, 7.25; N, 10.35.

3-{2-(2-Hydroxyethoxy)ethyl}-6-(4-chlorophenyl)-7-phenyl-3H-pyrrolo[2,3-*d*] pyrimidin-4(7H)-one 3d

Yield (64%); m.p.278-279^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1680 (CO), 3422 (OH);^{1}H NMR (DMSO) δ ppm: 2.39 (s, 1H, OH), 3.08-3.19 (t, 2H, N-CH_2), 3.43-3.57 (d, 2H, O-CH_2CH_2OH), 3.61-3.70 (d, 2H, N-CH_2CH_2), 3.77-3.84(t, 2H, CH_2OH), 6.90 (s, 1H, $H_{pyrrole}$), 7.25-7.85 (m, 9H, $H_{arom.}$), 8.14 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 409 [M$^+$] (7.30),411 [M^{+2}] (5). Anal.Calcd. for $C_{22}H_{20}ClN_3O_3$ (409.78): C, 64.47; H, 4.92; Cl, 8.65; N, 10.25.Found: C, 64.75; H, 4.85; N, 10.45.

6-(4-Chlorophenyl)-3-(2,3-dihydroxypropyl)-7-phenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one 3e

Yield (71%); m.p.310-312^{0}C; IR (KBr) ν_{max} [cm^{-1}]: 1683(CO), 3428(OH); ^{1}H NMR (DMSO) δ ppm: 2.50 (s, 1H, OH), 2.71-2.73(d, 2H, N-CH_2), 2.87-2.89(d, 2H, OH-CH_2), 3.33-3.42(m, 1H, CH), -6.84(s, 1H, $H_{pyrrole}$), 7.18-7.86(m, 9H, $H_{arom.}$), 8.04 (s, 1H, $H_{pyrimidine}$); ^{13}C NMR (DMSO) δ ppm : 158.06, 149.54, 144.44, 135.78, 134,29. 132.24, 130.06, 129.83, 128.98, 128.32, 128.26, 128.07, 120,58, 108.48, 103.12, 63.71, 61.90, 45.56; MS m/z [%]: 395.05 [M$^+$] (16.96).397.20[M^{+2}] (11.28). Anal.Calcd. For $C_{21}H_{18}ClN_3O_3$ (395.84): C, 63.72; H, 5.58; Cl, 8.96; N, 10.62.Found: C, 63.95; H, 5.35; Cl, 8.84; N, 10.35.

6-(4-Chlorophenyl)-3-nonyl-7-phenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)-one 3f

Yield (68%); m.p.280-282^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1681 (CO); ^{1}H NMR (DMSO) δ ppm: 0.74-0.83 (t, 3H, CH_3), 1.28-1.66 (m, 10H, $5CH_2$), 2.49-2.58 (m, 2H, N-CH_2CH_2), 3.13-3.44 (t, 2H, N-CH_2), 6.88 (s, 1H, $H_{pyrrole}$), 7.10-7.4 8 (m, 9H, $H_{arom.}$), 8.19 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 447 [M$^+$] (25.90). Anal.Calcd. for $C_{27}H_{30}ClN_3$ O (448): C, 72.39; H, 6.75; Cl, 7.91; N, 11.19.Found: C, 72.45; H, 6.25; Cl, 8.11; N, 11.25.

3-{2-(2-Hydroxyethoxy)ethyl}-6-(4-bromophenyl)-7-phenyl-3H-pyrrolo[2,3-*d*]pyrimidin-4(7H)one 3g

Yield (72%); m.p.316-317^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1689 (CO), 3420(OH); ^{1}H NMR (DMSO) δ ppm: ^{1}H NMR ($CDCl_3$) δ ppm: 2.31 (s, 1H, OH), 3.07-3.15 (t, 2H, N-CH_2), 3.45-3.56 (d, 2H, O-CH_2CH_2OH), 3.61-3.70 (d, 2H, N-CH_2CH_2), 3.75-3.80 (t, 2H, CH_2OH), 6.83 (s, 1H, $H_{pyrrole}$), 7.23-7.86 (m, 9H, $H_{arom.}$), 8.13 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 454.30 [M$^+$] (73.96).Anal.Calcd. For $C_{22}H_{20}BrN_3O_3$ (454.32): C, 58.16; H, 4.44; Br, 17.59; N, 9.25.Found: C, 58.55; H, 4.35; Br, 17.84; N, 9.37.

6-(4-Bromophenyl)-3-(2,3-dihydroxypropyl)-7-phenyl-3H-pyrrolo[2,3-d]pyrimidin-4(7H)-one 3h

Yield (78%); m.p.304-306^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1683 (CO), 3408(OH); ^{1}H NMR (DMSO) δ ppm: 2.48 (s, 1H, OH), 2.69-2.73 (d, 2H, N-CH_2), 2.84-2.89 (d, 2H, OH-CH_2), 3.30-3.46 (m, 1H, CH), 6.82 (s, 1H, $H_{pyrrole}$), 7.17-7.87 (m, 9H, $H_{arom.}$), 8.12 (s, 1H, $H_{pyrimidine}$); MS m/z [%]: 439 [M$^+$] (36.80).Anal.Calcd. For $C_{21}H_{18}BrN_3O_3$ (440.29): C, 57.29; H, 4.12; Br, 18.15; N, 9.54.Found: C, 57.55; H, 4.35; Br, 18.44; N, 9.37.

6-(4-Bromophenyl)-3-nonyl-7-phenyl-3H-pyrrolo [2,3-*d*]pyrimidin-4(7H)-one 3i

Yield (78%); m.p.304-306^{0}C; IR (KBr) ν_{max} [cm^{-1}]:1686 (CO); ^{1}H NMR (DMSO) δ ppm: 0.70-0.82 (t, 3H, CH_3), 1.22–1.64 (m, 10H, $5CH_2$), 2.48-2.55 (m, 2H, CH_2), 3.11-3.41 (t, 2H, N-CH_2), 6.89 (s, 1H,$H_{pyrrole}$), 7.10-7.45 (m, 9H, $H_{arom.}$), 8.18 (s, 1H, $H_{pyrimidine}$); ^{13}C NMR (DMSO) δ ppm : 207.69, 206.64, 186.81, 168.81, 131.24, 130.47, 130.08, 129.20, 128.20, 125.65, 120.55, 108.56, 103.43, 61.01, 58.75, 45.45, 43.02, 32.47, 31.21, 28.55, 25.88, 22.01, 13.86; MS m/z [%]: 491.10 [M$^+$] (38.72), 493 [M^{+2}] (9.35).Anal.Calcd. For $C_{27}H_{30}BrN_3O$ (492.45): C, 65.85; H, 6.14; Br, 16.23; N, 8.53.Found: C, 65.55; H, 6.35; Br, 16.43; N, 8.31.

3. Results and Discussion

Chemistry

6-Substituted-7-phenyl-3,7-dihydropyrrolo [2,3-*d*] pyrimidin-4-one derivatives **2a-c,** the key intermediate for synthesis of title compounds **3a-i** (Scheme 1) was prepared by addition of formic acid to **1a-c** and refluxed the mixture for 4 hr. The IR spectrum of **2a** showed absorption bands at 3427 & 1668 cm^{-1} assigned to imino (NH) and carbonyl (CO) groups respectively. Furthermore, its ^{1}H NMR spectrum showed a singlet at δ 8.10 ppm & 10.10 ppm indicated the formation of pyrimidine ring and imino (NH) respectively confirmed the structure. Mass spectrum of **2a** showed molecular ion peak at m/z 287 corresponding to its molecular formula $C_{18}H_{13}N_3O$.

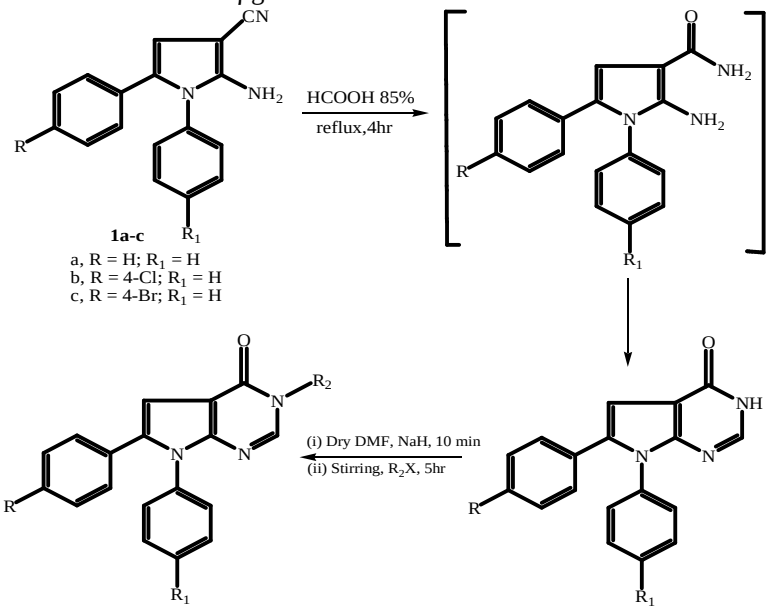
Compounds **2a-c** in dry DMF added sodium hydroxide and the mixture stirred at room temperature for 20 min and then the alkyl halide was added and refluxed to afford **3a-i**. Completion of the reaction was monitored by TLC. The title compounds **3a-i** was obtained in good yields (64 – 78%). The IR

spectrum of **3c** revealed the absence of the imino group (NH) and the appearance of absorption band at 1684 cm^{-1} for (CO) only. It's ^1H NMR spectrum also showed two triplet bands at δ 0.71–0.89 ppm & δ 3.19–3.43 ppm for methyl group (CH_3) and (N-CH_2) respectively. Also, the presence of characteristic multiplet at δ 1.20–1.67 ppm (5 CH_2) and δ 2.45–2.59 ppm (CH_2). All structures were assigned by their mass spectrum and elemental analysis.

Biology

Antiviral activity: There are many compound that synthesized, were shown to inhibit replication of both NDV virus and H5N1 virus in SPF (ECE's) when compared with "Ribavirin" (reference antiviral drug). The compounds **1a-c, 2a, 2c** showed good most anti Newcastle (ND) activating epically **1a,2a** are the most potent with MIC 2.6 &2.7µgm /ml respectively as shown in Table (1). Also, compounds **3a, 3c, 3g, 3h, 3i** showed a good anti (H5N1) where **3a, 3b, 3g, 3h,** are highly potent with MIC 3.8 , 3.6 , 4.5 and 4.1 µgm /ml respectively as shown in Table (2) .

Antibacterial activity: The in vitro antibacterial activating of different synthesized compound against two gram-negative bacteria [*Echerichia coli* ATCC 25992, *Salmomella typhimurium* ATCC 14028] were exhibited various levels of antibacterial effect against tested bacterial strains, as compared with reference antibiotic "Suttrium" where they produce a large and clear zone of inhibition when sensitive, organisms come in contact with the susceptible antimicrobial compound **1a-c, 2a-c, 3a-b, 3g-h** showed antibacterial effect where **1a, 1b, 2a, 2b** are the most potent compounds with MIC 2.6, 2.9, 2.8, 3.2 µgm/ml respectively as shown in table (4).



1a-c
a, R = H; R_1 = H
b, R = 4-Cl; R_1 = H
c, R = 4-Br; R_1 = H

3a-i
a, R = H; R_1 = H; R_2 = -$CH_2CH_2OCH_2CH_2OH$
b, R = H; R_1 = H; R_2 = -$CH_2CHOHCH_2OH$
c, R = H; R_1 = H; R_2 = -$(CH_2)_8CH_3$
d, R = 4-Cl; R_1 = H; R_2 = -$CH_2CH_2OCH_2CH_2OH$
e, R = 4-Cl; R_1 = H; R_2 = -$CH_2CHOHCH_2OH$
f, R = 4-Cl; R_1 = H; R_2 = -$(CH_2)_8CH_3$
g, R = 4-Br; R_1 = H; R_2 = -$CH_2CH_2OCH_2CH_2OH$
h, R = 4-Br; R_1 = H; R_2 = -$CH_2CHOHCH_2OH$
i, R = 4-Br; R_1 = H; R_2 = -$(CH_2)_8CH_3$

2a-c
a, R = H; R_1 = H
b, R = 4-Cl; R_1 = H
c, R = 4-Br; R_1 = H

Scheme 1. Synthesis of pyrrolo[2,3-*d*]pyrimidine derivatives **3a-i**

Conclusion

In summery, we have described an efficient synthesis for preparation of new pyrrolo[2,3-*d*]pyrimidine derivatives .These products were evaluated in vitro for their antiviral and antibacterial activities. It is good to say that the compounds **1a-c, 2a-c,** are shown highly antiviral activity towards Newcastle Disease Virus (NDV) where **1a, 2a** are highly potent.

Also compounds **3a-d, 3g-i** are shown highly antiviral activity towards Avian Influenza virus (H5N1) where **3a, 3b, 3g, 3h** are highly potent.

And it is good to be improved antibacterial activity was observed for most of the compounds against gram-negative bacteria used in the study.

Especially the compounds **1a-c, 2a-b, 3a, 3g, 3h** are shown highly antibacterial activity.

Finally, the outcome of these study is the possibility of using of such pyrrolo[2, 3-*d*]pyrimidines derivatives in the production of effective antiviral drug and in vaccine production after applying the known roles.

It is the first record to use these new compounds against highly pathogenic avian influenza virus (H5N1) causing "Bird Flu", and against Newcastle Disease Virus (NDV).

Corresponding author
Khaled M. Hassan Hilmy
Chemistry Department, Faculty of Science, El-Menofia University, Shebin El-Kom, Egypt.
hilmykhaled@yahoo.com,
khaledhilmy@hotmail.com

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