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Synthesis, toxicological and biochemical studies of new heterocyclic compounds derived from acetanilide and pyrrole derivatives

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Abstract

Synthesis of 2,5-diamino-oxazole (2a), 2,5-diamino-thiazole (2b), 2,4-diamino-*5H*-imidazole (3) derivatives were achieved through reactions of chloroacetanilide (1) with urea, thiourea and guanidine, respectively. Compounds 5a, b were also prepared. Compound 6 was obtained upon heating of 5a with acetic anhydride in presence of acetic acid. We have used the *ANRORC* approach to obtain compounds 7a, b and 8. Reactions of arylidene-malononitriles or (z)-ethyl-2-cyano-3-arylacrylates with 8 gave compounds 9a-c and 10a-c. Compounds 11a-c was obtained via cyclization of 8 with aldehyde and thiourea.

Biological studies were undertaken to asses the effect of some these new compounds on altered biochemical parameters in deltamethrin-exposed rats. Blood cholinesterase activity was significantly inhibited, 25% and 15% for plasma and erythrocyte, after rat treatment with deltamethrin for one month.

Key words: Pyrrole, indene, pyrimidine, deltamethrin, cholinesterase

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Introduction

Polyfunctional derivatives of aminopyrroles constitute an important family of compounds due to their wide applications as antibacterial, antiviral, anticonvulsant, analgesic, anti-inflammatory and antipyretic activities [1-4]. Due to the importance of pyrrole derivatives for various applications, great efforts have been made toward the preparation of these heterocyclic systems [5-9].

Neurological activity of animals can be extremely sensitive to environmental contamination [10]. However, acetylcholine is one of the major neurotransmitters in the brain and is hydrolyzed by

enzyme acetylcholinaesterase. Morbus Alzheimer, a progressive, neurodegenerative disease, is characterized by selective loss of cholinergic neurons in the basal forebrain. Further pathological hallmarks are the formation of intracellular neurofibrillary tangles and extracelluler deposition of β-amyloidal protein [11]. Therefore, measurement of acetyl cholinesterase (AChE) activity is routinely used as a biomarker of exposure to certain groups of contaminants, such as pyrethroids [12]. These insecticides bind to the enzyme, leading to the accumulation of acetylcholine in the synapse, resulting in the disruption of normal nervous system function [13]. Deltamethrin is one of the more neurotoxic members of a relatively new and commonly used class of the pyrethroid insecticides [14].

Results and Discussion

Chemistry

Recently we described several efficient approaches to heteroaromatic systems using functionally substituted enamine precursors [15-18].

Therefore, we reported here the results of our work which aimed at exploring the potential utility of 2-amino-1- (2-methyl-4-nitro-phenyl)-5-oxo-4, 5-dihydro-*1H*-pyrrole-3-carboxylic acid ethyl ester in the heterocyclic syntheses.

The most straightforward protocol to synthesize 2, 5-diamino-oxazole (2a), 2, 5-diamino-thiazole (2b) and 2, 4-diamino- *5H*-imidazole (3) , derivatives was through the reaction of 2-chloro-N-(2- methyl-4-nitro-phenyl)- acetamide (1) [19]with urea, thiourea and guanidine respectively (scheme 1, exp.).

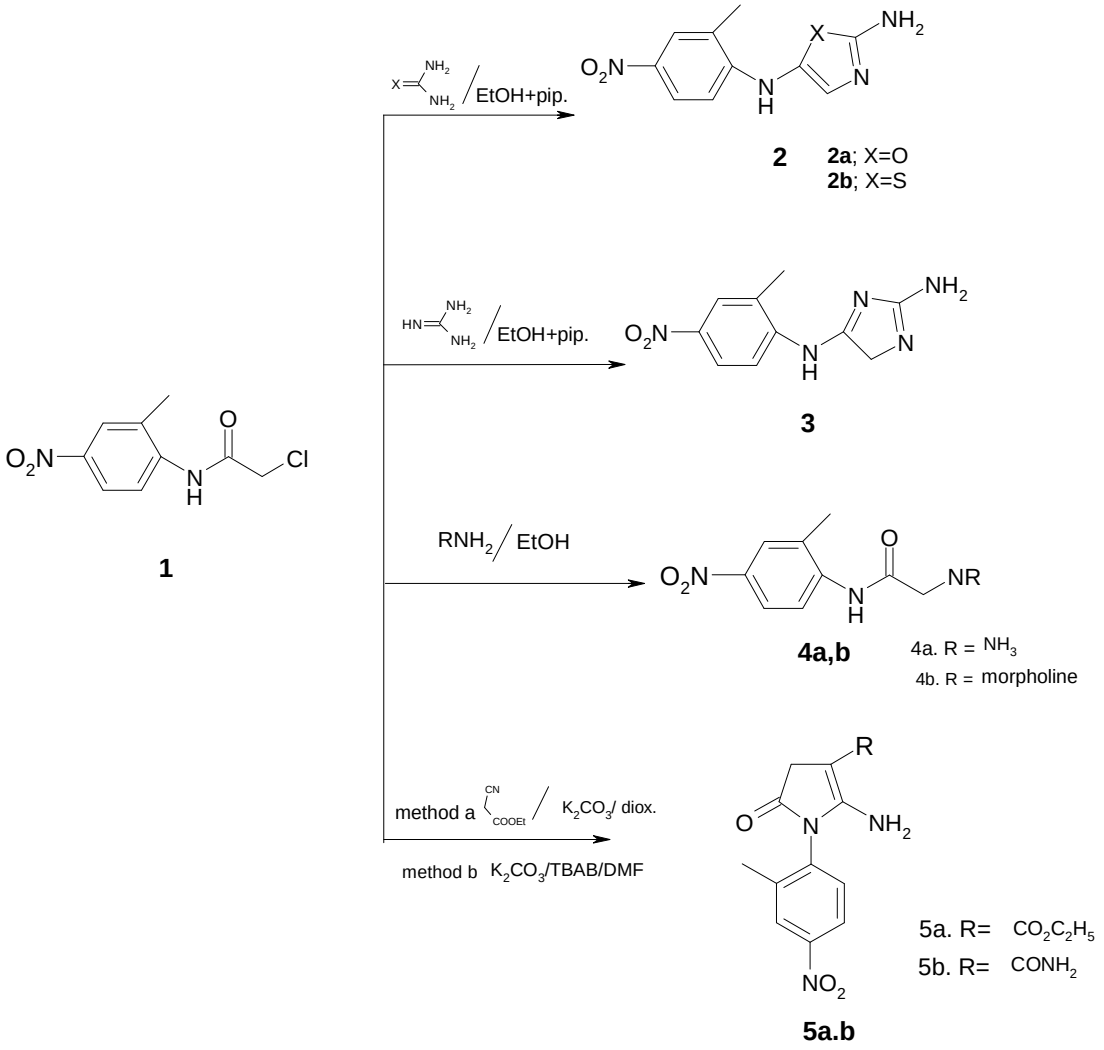
The assignment of structures of the newly synthesized products were achieved from their correct values in elemental analyses, IR showed absorption peaks at (KBr, ν/cm^{-1}): ranged from 3408 to3325 (NH_2) for 2a, b and 3. ^{1}H-NMR (DMSO-d_6): δ/ppm revealed signals at 7.55(s, 1H, oxazole), 8.01(s, 1H, thiazole) and 2.62 (s, 2H, CH_2 imidazol) derivatives respectively in addition to their agreeable features in M.S (E.I) m/z %.

Compounds 4a, b were prepared via neuclophillic displacement of halogen and their structures confirmed from their correct values in elemental analyses and their agreeable data in IR, ^{1}H-NMR and mass spectroscopy (scheme 1,exp.).

2-Amino-1-(2-methyl-4-nitro-phenyl)-5-oxo-4,5-dihydro-*1H*-pyrrole-3-carboxylic acid ethyl ester and the carboxamide derivatives (5a,b) were synthesized by following Gewald one pot ring closure reaction [20].

Unfortunately, this method provided us from low to moderate yield. Therefore, a novel phase transfer catalyses strategy was used (scheme 1, exp.).

The structure of the formed products were confirmed from their correct values in elemental analyses, IR spectra for 5a showed peaks at (KBr, ν/cm^{-1}): 3426-3385(NH_2), 1702, 1650 (2CO). ^{1}H-NMR (DMSO-d_6): δ/ppm gave characteristic signals at δ 1.53 (t, 3H, CH_3), 4.28 (q, 2H, CH_2), 5.20(s, 2H, CH_2, pyrrole) and M.S (E.I) m/z% 232(M^+ -$CO_2C_2H_5$, 43%), 65 (100).



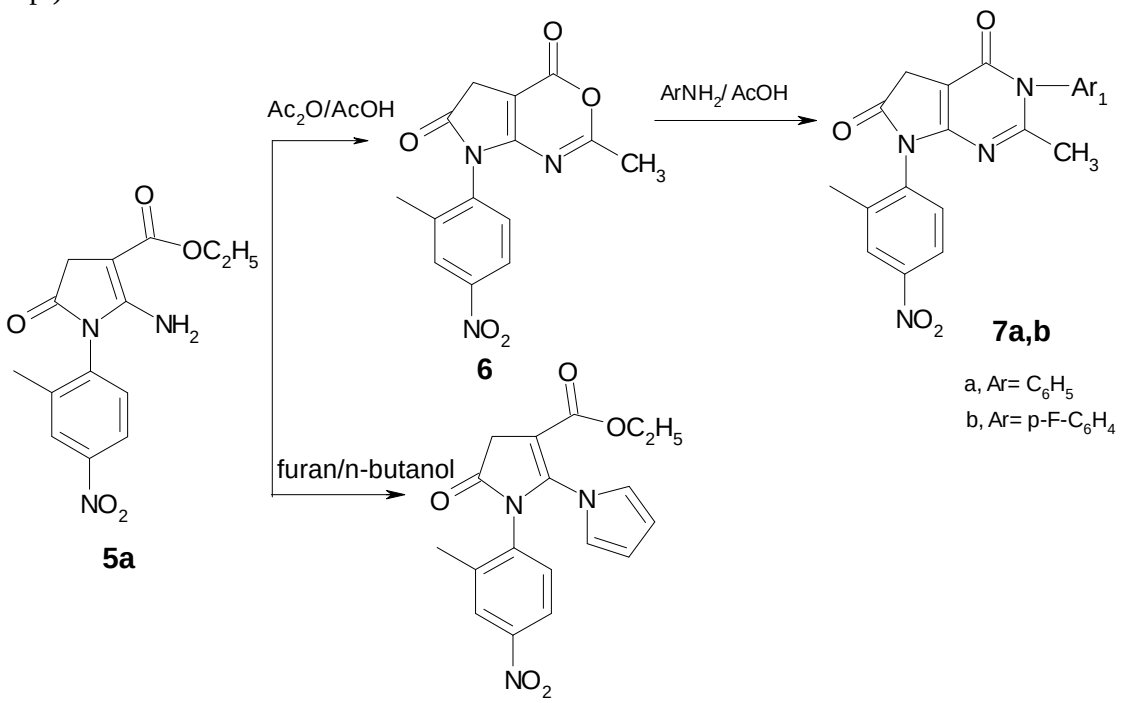
Scheme 1

Upon heating of **5a** with acetic anhydride in presence of acetic acid, 1, 3-dihydro-5-oxa-1, 7-diaza-indene-2, 4-dione derivative (**6**) was obtained. The structure of the indenedione derivative was confirmed from its correct values in elemental analyses and its combatable data of IR, ^{1}H-NMR and Mass spectra (scheme 2, exp.).

ANRORC processes (consisting of an initial *A*ttack of *N*ucleophile followed by *R*ing-*O*pening and *R*ing-*C*losure) have been extensively studied by VanderPlas and his coworkers [21,22] which are represent a useful tool in the hand of the synthetic heterocyclic chemist to achieve the ring transformation of heterocyclic systems.

We have recently used the *ANRORC* approach as valuable method for the obtainment of ring transformation of heterocyclic systems [23]. 7-Arylpyrrolo [2, 3-*d*]pyramidine- dione derivatives (7a,b) and [1, 2$^{\backslash}$] bipyrrolyl-3$^{\backslash}$-carboxylic acid ethyl ester (8) were prepared depending upon this finding in good yields.

The structure of bipyrrole (8) derivative was achieved from its agreeable data of elemental analyses and in addition to its combatable data of IR, ^{1} H-NMR and mass spectra. IR for compound 8 showed peaks at (KBr, ν/cm^{-1}): 1686, 1672 (2CO) and ^{1} H-NMR revealed signals characteristics for pyrrole ring at 7.93(d, 2H, *j*= 4.06Hz) and 8.84(d, 2H, *j*= 4.06 Hz,) (scheme 2, exp.).



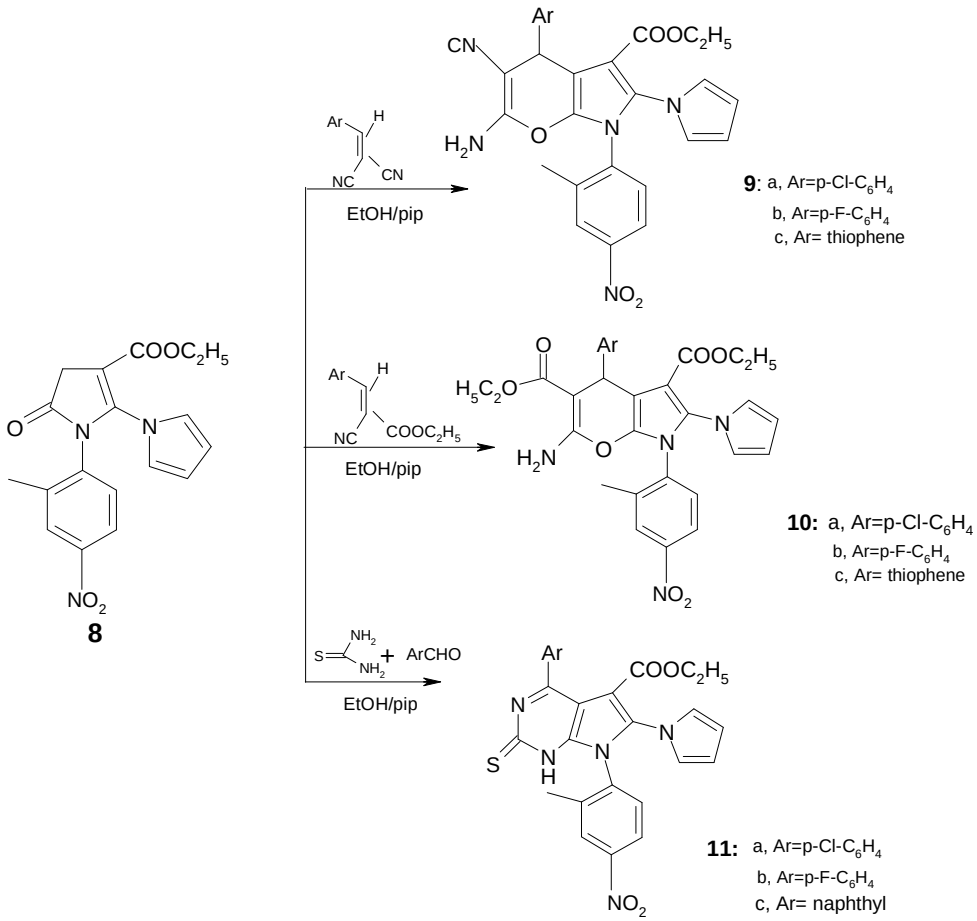
Scheme 2

Elnagdi and his coworkers reported the first addition of cinnamonitrile to cyclic active methylene compounds [24-26].

Therefore, reactions of arylidene-malononitriles or (z)-ethyl-2-cyano-3-arylacrylates with active methylene cyclic ketone (8) gave 2-amino-3-cyano-pyrrol-1-yl-dihydro- pyrano [2, 3-*b*]pyrrole-5-carboxylic acid ethyl ester (9a-c) derivatives and 2-amino-pyrrol-1-*yl*-dihydro-pyrano[2,3-*b*]pyrrole-3,5-dicarboxylic acid diethyl ester (10a-c) derivatives, respectively.

Compounds 9a-c were characterized by the presence of CN absorption peaks in IR at (KBr, ν/cm^{-1}): 2215(CN) and significant signals at δ/ppm (DMSO-d_6, δ/ ppm): 3.27, 3.20, 2.95 (s, 2H, NH_2, exchangeable with D_2O) and 6.24, 6.24, 6.65 (s, 1H, pyrane), in addition to their agreeable elemental analyses and Mass spectra (scheme 3, exp.).

Compounds 10a-c were elucidated from their combatable data in elemental analyses, IR, ^{1}H-NMR and Mass spectra (scheme3, exp.).
Finally, we applied the Beganillie method [27] to obtain new series of heterocyclic compounds with pyrimidine nucleus. The structure of the pyramidine derivatives were confirmed from their correct values in elemental analyses and their combatable data of IR, ^{1}H-NMR and Mass spectra (scheme 3, exp.).



Scheme 3

Biochemical studies

i. Effect on cholinesterase activity

Data represented in table 1 showed that, treatment of rats with deltamethrin caused significant decreased in the activity of acetyl cholinesterase in both plasma and erythro- cytes, especially in plasma ChE (25% inhibition) as compared with control groups. The present study indicated that treatment with compounds 4a and 9c alone did not cause any significant change in the enzyme activities, while these two compounds in combination with deltamethrin alleviated its negative effect on the activities of the cholinesterase enzymes. Compound 9c seems to be more effective than compound 4a as a detoxification compound as shown in table 1.

These compounds may act as chemicals which can reverse the binding of choline- sterase inhibitors with acetyl cholinesterase. They attached to the cholinesterase inhibitor and removed it from cholinesterase, allowing the enzyme to work normally again. The present study is in agreement with the finding of [28].

ii. Effect on some enzyme activities

Table 2 showed the activities of liver and antioxidant enzymes. In case of deltamethrin group, there was a moderate elevation in ALT, AST and ALP reached to more than 30% as compared to the control animals. While, group of animals treated with compounds 4a and 9c alone and in combination with deltamethrin showed no significant elevation at the end of the experimental period as compared with the control one.

Results also indicated that catalase and glutathione-S-transferase enzyme activities were significantly decreased in serum of rats treated with deltamethrin (table 2). On the other hand, rats treated with compound 4a and 9c with deltamethrin, recorded no significant difference between treated and control group.

Data represented in table 3 showed that treatment of compounds 4a and 9c with deltamethrin caused significant decrease in serum total protein and albumen, and showed increase in urea and creatinine concentration (to more than 40%) compared to control animals.

The presence of compounds 4a and 9c with deltamethrin counteracted its hazardous effect.

iii. Lipid profile

Table 4 showed that the concentration of cholesterol, triglycerides, and low density lipoprotein were significantly increased by deltamethrin treatment, while high density lipoprotein levels were decreased as compared to control group. However, treatment with 4a and 9c minimized the toxic effects of deltamethrin and reduced the elevation in serum lipids.

The presence of 4a and 9c with deltamethrin alleviated its toxic effect on most of the measured parameters (tables 1-4).

From the present results, we can concluded that exposure of animals to deltamethrin was capable of inducing marked hazardous alterations in some biochemical parameters. Using compounds 4a and 9c could have capability to alleviate the harmful effect of deltamethrin.

Several studies have indicated that reactive oxygen species have bean implicated in the toxicology of pyrethroids [29], so the protective effect of 4a and 9c observed in our study, could be important for protecting the different tissues against the oxidative injury following the use of deltamethrin.

Materials and Methods

Chemistry

The purity of the synthesized compounds was evidenced by TLC and their elemental analyses were generally found to be within ± 0.04% of the theoretical values.IR spectra (KBr, νcm $^{-1}$) were recorded on Perkin Elmer 580 spectrophotometer. ^{1}H-NMR spectra were carried on JNM, FT- NMR-EX270, run ^{1}H-NMR 270 MHz, in DMSO-d_6 using TMS as internal standard and chemical shifts are expressed in δ. ppm. Mass spectra were recorded on Varian Mat 112 spectrometer.

2-Chloro-N-(2- methyl-4-nitro-phenyl)- acetamide (**1**) [19].

General procedure for preparation of 2a, b and 3:
A solution of an equimolecular amounts of compound 1 (2.28g, 10mmole) and urea, thiourea, guanidine hydrochloride, respectively, in absolute ethanol (20 ml) containing (1ml) of pipredine was heated under reflux for 12 hs. The solid product collected by filtration and crystallized from ethanol to give 2a, b, and 3.

N^*5^*-(2-methyl-4-nitro-phenyl)-oxazole-2, 5-diamine ***(2a):***
(53% yield), m.p= 257-9 ^oC. Analyses calculated**:** $C_{10}H_{10}N_4O_3$ (234.22); IR (KBr, ν/cm^{-1}): 3408-3325 (NH_2), 3228(NH); ^{1}H-NMR (DMSO-d_6): δ/ppm= 2.37(s, 3H, CH_3), 4.06 (s, 2H, NH_2, exchangeable with D_2O), 7.55(s, 1H, oxazole), 7.59(d, 2H, *j* = 8.6Hz, Ar-H), 7.98 (s, H, Ar-H), 8.32(bs, 1H, NH, exchangeable with D_2O); M.S (E.I) m/z% =228(M^+ -NH_2, 56%), 66 (100).

N^*5^*-(2-methyl-4-nitro-phenyl)-thiazole-2, 5-diamine ***(2b):***
(60% yield), m.p= 215-7^oC. Analyses calculated**:** $C_{10}H_{10}N_4O_2S$ (250.28); IR (KBr, ν/cm^{-1}): 3416-3340 (NH_2), 3292(NH); ^{1}H-NMR (DMSO-d_6): δ/ppm= 2.35(s, 3H, CH_3), 4.02 (s, 2H, NH_2, exchangeable with D_2O), 7.59(d, 2H, *j* = 8.6Hz, Ar-H), 7.98 (s, H, Ar-H), 8.01(s, 1H, thiazole), 8.32(bs, 1H, NH, exchangeable with D_2O); M.S (E.I) m/z% =250(M^+ -NH_2, 40%), 66 (100).

N^*4^*-(2-methyl-4-nitro-phenyl)-5H-imidazole-2, 4-diamine ***(3):***
(63% yield), m.p=230-2 ^oC. Analyses calculated**:** $C_{10}H_{11}N_5O_2$ (250.28); IR (KBr, ν/cm^{-1}): 3420-3362 (NH_2), 3252(NH); ^{1}H-NMR (DMSO-d_6): δ/ppm=2.62(s, 2H, CH_2), 2.35(s, 3H, CH_3), 4.02 (s, 2H, NH_2, exchangeable with D_2O), 7.59 (d, 2H, *j* = 8.6Hz, Ar-H), 7.98 (s, H, Ar-H), 8.32 (bs, 1H, NH, exchangeable with D_2O); M.S (E.I) m/z% =236(M^+ -NH_2, 40%), 66 (100).

N-(2-methyl-4-nitro-phenyl)-acetamide derivatives (4a, b):
General procedure:
A solution of an equimolecular amounts of compound **1** (2.28g, 10mmole) and hydrazine hydrate or morpholine in absolute ethanol (20 ml) was stirred at room temperature for 8 hs. The solid product collected by filtration and crystallized from ethanol to give **4a, b**.

4a: Yield %= 90; mp= 138-40^{0}C., Analyses calculated**:** $C_9H_{12}N_4O_3$ (224.22); IR (KBr, ν/cm^{-1}): 3372-3332 (NH_2), 3127(NH); ^{1}H-NMR (DMSO-d_6): δ/ppm=2.35(s, 3H, CH_3), 3.21(s, 2H, CH_2), 6.32. (s, 2H, NH_2, exchangeable with D_2O), 7.49(d, 2H, *j* = 8.6Hz, Ar-H), 7.94 (s, H, Ar-H), 8.04, 8.54 (2bs, 2H, 2NH, exchangeable with D_2O).

4b: Yield %= 65; mp= 240-2^oC. Analyses calculated**:** $C_{13}H_{17}N_3O_4$ (279.33); ^{1}H-NMR (DMSO-d_6): δ/ppm=2.15(s, 3H, CH_3), 2.24 (m, 4H, morpholine), 3.33 (m, 4H, morpholine), 3.41(s, 2H, CH_2), 7.69(d, 2H, *j* = 8.6Hz, Ar-H), 7.94 (s, H, Ar-H), 8.51 (bs, H, NH, exchangeable with D_2O).

2-Amino-1-(2-methyl-4-nitro-phenyl)-5-oxo-4, 5-dihydro-1H-pyrrole derivatives **(5a, b):**
Method A:
To a solution of chloroacetanilide **1**(2,28g, 10mmole) in 30 ml dioxane, ethylcyano acetate or cyanoacetamide (12momole) and 2-3 drops of piperidine were added. The reaction mixture was heated under reflux for 3 hrs. Then it was cooled to room temperature. Where100 ml of water

was added. The formed solid products collected by filtration and crystallized from isoprpanol affording 5a, b.

Method B:
To a mixture of anhydrous potassium carbonate (3 g), dry DMF (40ml), chloroacetanilide **1**(2.28g, 10mmole) and catalytic amounts of tetrabutylammonium bromide (TBAB), ethyl cyanoacetate or cyanoacetamide (12 mmole) was added. The reaction mixture was vigorously stirred at 60^{o} C., the reaction was followed by TLC. After completion of reaction, it was then cooled to room temperature. Where100 ml of water was added. The formed solid collected by filtration and crystallized from ethanol affording 5a, b.

5a: Yield %= 65; mp= 195-200^{o}C., Analyses calculated**:** $C_{14}H_{15}N_3O_5$ (305.29); IR (KBr, ν/cm^{-1}): 3426-3385(NH_2), 1702, 1650 (2CO); ^{1}H-NMR (DMSO-d_6): δ/ppm =1.53 (t, 3H, CH_3), 2.38 (s, 3H, CH_3), 3.23 (s, 2H, NH_2, exchangeable with D_2O), 4.28 (q, 2H, CH_2), 5.20 (s, 2H,CH_2, pyrrole), 7.22 (d, 2H, *j* = 8.2Hz, Ar-H), 7.93 (s, 1H, Ar-H), M.S (E.I) m/z% =232(M^+ -$CO_2C_2H_5$, 43%), 65 (100).

5b: Yield %= 57; mp= 107-9^{o}C**.,** Analyses calculated**:** $C_{12}H_{12}N_4O_4$ (276.25); IR (KBr, ν/cm^{-1}): 3432-3390(NH_2), 1683, 1650 (2CO); ^{1}H-NMR (DMSO-d_6): δ/ppm =2.18 (s, 3H, CH_3), 6.23, 8.03 (2s, 4H, 2NH_2, exchangeable with D_2O), 5.34 (s, 2H, CH_2, pyrrole), 7.25 (d, 2H, *j* =8.2Hz, Ar-H), 7.97 (s, 1H, Ar-H),

***6-Methyl-1- (2-methyl-4-nitro-phenyl)-1, 3-dihydro-5-oxa-1, 7-diaza-indene-2, 4*-dione (6):**
A solution of 5a in glacial acetic acid (15ml) and acetic anhydride (15ml) was heated under reflux for 10 hs. The excess solvent evaporated under vacuum, the resulted oil treated with methanol (20 ml). The solid formed filtered off and crystallized from methanol to give 6 in 46% yields with mp 250^{o}C.
Analyses calculated**:** $C_{14}H_{11}N_3O_5$ (301.26); IR (KBr, ν/cm^{-1}), 1702, 1680(2CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.32, 2.41(2 s, 6H, 2CH_3), 5.32(s, 2H, CH_2 pyrrole), 7.57(d, 2H, *j* = 10.2Hz, Ar-H), 7.94 (s, 1H, Ar-H), M.S (E.I) m/z% =302(M^{+1}, 63%), 65 (100).

***2-Methyl-1-(2-methyl-4-nitro-phenyl)-7-aryl-5,7-dihydro-3H-pyrrolo[2,3-d]- pyrimmidine-4,6-dione* (7a):**
To a solution of compound **6** (6.02 g, 20 mmole) in acetic acid (20 ml), aniline and *p*-floroanilne respectively (25 mmole) was added. The reaction mixture was heated under refluxing temperature for 3 hs. The reaction mixture cooled and poured onto crushed ice with stirring. A precipitate solid separated, filtered off and washed with water, dried and crystallized from appropriate solvents to give **7a, b**.

7a: Crystallized from ethanol, Yield: 55%, mp. 237-9^{o}C., Analyses calculated**:** $C_{20}H_{16}N_4O_4$ (376.38); IR (KBr, ν/cm^{-1}), 1689, 1670(2CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.34, 2.30(2 s, 6H, 2CH_3), 5.02(s, 2H, CH_2 pyrrole), 7.57-8.01(m, 8H, Ar-H); M.S (E.I) m/z% =377(M^{+1}, 63%), 65 (100).

2-Methyl-1-(2-methyl-4-nitro-phenyl)-7-(4-floro-phenyl-5,7-dihydro-3H-pyrrolo[2,3d]pyrimi dine-4,6-dione (7b):
Crystallized from isopropanol.
Yield: 42% , mp. 212-4°C., Analyses calculated**:** $C_{20}H_{15}$ FN_4O_4 (394.37); H-NMR (DMSO-d_6): δ/ppm=1.39, 2.42(2 s, 6H, $2CH_3$), 4.49(s, 2H, CH_2 pyrrole), 7.38-8.00(m, 7H, Ar-H); M.S (E.I) m/z% =395(M^{+1}, 36%), 396 (M^{+2}, 31 %), 74 (100).

1`-(2-Methyl-4nitro-phenyl)-5`-oxo-4`, 5`-dihydro-1`H-[1, 2`] bipyrrolyl-3`-carboxylic acid ethyl ester (8):
To a solution of compound **5a** (6.02 g, 20 mmole) in n- butanol (20 ml), furane (1.70g, 25 mmole) was added. The reaction mixture was heated under refluxing temperature for 3 hs. The reaction mixture cooled and poured onto crushed ice with stirring. A precipitate solid separated, filtered off and washed with water, dried and crystallized from ethanol.

Yield = 63%., mp 224-6°C; IR (KBr, ν/cm^{-1}): 1686, 1672 (2CO); Analyses calculated**:** $C_{20}H_{15}$ FN_4O_4 (394.37); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.53 (t, 3H, *j*=5.40, CH_3), 2.36 (s, 3H, CH_3), 4.21(q, 2H, *j*= 5.32, CH_2), 4.49 (s, 2H, CH_2 pyrrole), 7.32 (d, 2H, *j*= 10.8 Ar-H), 7.93 (d, 2H, *j*= 4.06Hz, pyrrole), 8.32 (s, 1H, Ar-H), 8.84 (d, 2H, *j*=4.06Hz, pyrrole); M.S (E.I) m/z% =355(M^+, 23%), 76 (100).

General procedure for preparation of 9a-c, 10a-c
An equimolecular amounts of 8 with arylidene-malononitriles or (z)-ethyl-2-cyano-3-arylacrylates (*p*-chlorophenyl, *p*-F-phenyl, thiophene-derivatives), respectively in ethanol (15ml) containing 1ml of piperidine was heated under reflux for 10 hs. The excess solvent evaporated under vacuum, the resulted solid filtered off, washed with petroleum ether 40-60 and crystallized from appropriate solvent to give 9a-c, 10a-c.

2-Amino-4-(4-chloro-phenyl)-3-cyano-7-(2-methyl-4-nitro-phenyl)-6-pyrrol-1-yl-4,7-dihydro-pyrano[2,3-b]pyrrole-5-carboxylic acid ethyl ester ***(9a):***
Crystallized from ethanol
Yield = 58%; mp158-60 °C., Analyses calculated**:** $C_{28}H_{22}Cl$ N_5O_5 (543.93); IR (KBr, ν/cm^{-1}): 3852-3798 (NH_2), 2215(CN), 1686, (CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.34(t, 3H, *j*=5.40, CH_3), 2.46(s, 3H, CH_3), 3.27(s, 2H, NH_2 ,exchangeable with D_2O), 3.46(q, 2H, *j*= 5.32, CH_2), 6.24(s, 1H, pyrane),7.32-8.28(m, 7H, Ar-H), 7.93(d, 2H, *j*= 4.06Hz, pyrrole), 7.70 (d, 2H, *j*= 4.06 Hz, pyrrole); M.S (E.I) m/z% =543(M^+, 23%), 544 (M^{+1}, 19%), 76 (100).

2-Amino-4-(4-floro-phenyl)-3-cyano-7-(2-methyl-4-nitro-phenyl)-6-pyrrol-1-yl-4,7-dihydro-pyrano[2,3-b]pyrrole-5-carboxylic acid ethyl ester ***(9b):***
Crystallized from ethanol
Yield = (72%); mp180-2°C. Analyses calculated**:** $C_{28}H_{22}F$ N_5O_5 (527.51); IR (KBr, ν/cm^{-1}): 3840-3798 (NH_2), 2215(CN), 1686, (CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.54 (t, 3H, *j*=5.40, CH_3), 2.34 (s, 3H, CH_3), 3.20(s, 2H, NH_2 ,exchangeable with D_2O),3.46 (q, 2H, *j*= 5.32, CH_2), 6.24(s, 1H, pyrane),7.32-8.28 (m, 7H, Ar-H), 7.72(d, 2H, *j*= 4.06Hz, pyrrole), 8.01 (d, 2H, *j*= 4.06 Hz, pyrrole).; M.S (E.I) m/z% =527(M^+, 23%, M^{+1}528, 19%), 76 (100).

2-Amino-3-cyano-7-(2-methyl-4-nitro-phenyl)-6-pyrrol-1-yl-4-thiophen-2-yl-4,7-dihydro pyrano[2,3-b]pyrrole-5-carboxylic acid ethyl ester ***(9c):***
Crystallized from ethanol
Yield = (72%); mp125-7^{o}C. , Analyses calculated**:** $C_{26}H_{21}N_5O_5S$ (515.51); IR (KBr, ν/cm^{-1}): 3402-3368 (NH_2), 2215(CN), 1675, (CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.72(t, 3H, *j*=5.40, CH_3), 2.38(s, 3H, CH_3), 2.95(s, 2H, NH_2, exchangeable with D_2O), 3.42(q, 2H, *j*= 5.32, CH_2), 6.65(s, 1H, pyrane),7.52(d, 2H, *j*= 10.8 Ar-H), 7.65(d, 2H, *j*= 4.06Hz, pyrrole), 7.68(s, 1H, Ar-H), 8.23(d, 2H, *j*=4.06Hz, thiophen), 7.81(d, 2H, *j*= 4.06Hz, pyrrole), 8.13 (s, 1H, thiophen).
M.S (E.I) m/z% =515(M^+, 42%, M^{+1}516, 10%), 65 (100).

2-Amino-4-(4-chloro-phenyl) - 7-(2-methyl-4-nitro-phenyl)-6-pyrrol-1-yl-4, 7-dihydro - pyrano[2,3-b]pyrrole-3,5-dicarboxylic acid diethyl ester **(10a):**
Crystallized from ethanol
Yield = 72%; mp146-8 ^{o}C. Analyses calculated**:** $C_{30}H_{27}ClN_4O_7$ (591.03); IR (KBr, ν/cm^{-1}): 3898 (NH_2), 1686, 1673 (2CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.72(m, 6H, $2CH_3$), 2.46(s, 3H, CH_3), 3.01(s, 2H, NH_2 , exchangeable with D_2O), 4.02(m, 4H, $2CH_2$), 7.51-8.20(m, 7H, Ar-H), 7.67(d, 2H, *j*= 4.06Hz, pyrrole), 7.79 (d, 2H, *j*= 4.06 Hz, pyrrole), 8.34(s, 1H, pyrane); M.S (E.I) m/z% =592(M^{+1}, 30%), 593 (M^{+2}, 09%), 76 (100).

2-Amino-4-(4-floro-phenyl) - 7-(2-methyl-4-nitro-phenyl)-6-pyrrol-1-yl-4, 7-dihydro-pyrano[2,3-b]pyrrole-3,5-dicarboxylic acid diethyl ester **(10b):**
Crystallized from ethanol
Yield = 72%.162-4 ^{o}C. , Analyses calculated**:** $C_{30}H_{27}FN_4O_7$ (574.57); IR (KBr, ν/cm^{-1}): 3898 (NH_2), 1686, 1673 (2CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.52(m, 6H, $2CH_3$), 2.32(s, 3H, CH_3), 3.01(s, 2H, NH_2 , exchangeable with D_2O), 4.02(m, 4H, $2CH_2$), 7.45-8.20(m, 7H, Ar-H), 7.87(d, 2H, *j*= 4.06Hz, pyrrole), 8.01 (d, 2H, *j*= 4.06 Hz, pyrrole), 8.30(s, 1H, pyrane); M.S (E.I) m/z% =574(M^{+1}, 30%, M^{+2}575, 09%), 76 (100).

2-Amino-7-(2-methyl-4-nitro-phenyl)-6-pyrrol-1-yl-4-thiophen-2-yl-4,7- dihydro-pyrano[2,3-b]pyrrole-3,5-dicarboxylic acid diethyl ester **(10c):**
Crystallized from ethanol
Yield = (72%); mp137-9 ^{o}C. , Analyses calculated**:** $C_{28}H_{26}N_4O_7S$ (562.62); IR (KBr, ν/cm^{-1}): 3402 (NH_2), 1681, 1675, (2CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.92(m, 6H, *j*=5.40, CH_3), 2.34(s, 3H, CH_3), 4.05(m, 4H, *j*= 5.32, CH_2), 3.26(s, 2H, NH_2, exchangeable with D_2O), 7.40(d, 2H, *j*= 9.8 Ar-H), 7.62(d, 2H, *j*= 4.24Hz, pyrrole), 7.80(s, 1H, Ar-H), 7.83(d, 2H, *j*= 4.24Hz, pyrrole), 8.02(d, 2H, *j*=5.20Hz, thiophen), 8.13 (s, 1H, thiophen), 9.01(s, 1H, pyrane); M.S (E.I) m/z% =562(M^+, 22%), 563 (M^{+1}, 10%), 65 (100).

General preparation of 11a-c
An equimolecular amounts of 8 and appropriate aldehyde(*p*-chlorobenzaldehyde, *p*-florobenzaldhyde and naphthadehyde) and thiourea in absolute ethanol (30ml) in presence of acetic acid (10ml) was refluxed for 15hs. The reaction mixture was poured onto cold water (150 ml), filtered off, washed with petroleum ether 60-80 and finally crystallized from the appropriate solvent.

4-(4-chloro-phenyl)-7-(2-methyl-4-nitro-phenyl)-6-pyrrol-1-yl-2-thioxo-2,3,4,7, tetra- hydro-1H-pyrrolo[2,3-d]pyrimidine-5-carboxylic acid ethyl ester **(11a):**
Crystallized from ethanol
Yield = 60%. mp 242-4 ^{o}C., Analyses calculated**:** $C_{26}H_{22}ClN_5O_4S$ (536.01); IR (KBr, ν/cm^{-1}): 2954 (NH), 1675, (CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.78 (t, 3H, *j*=5.40, CH_3), 2.37 (s, 3H, CH_3), 4.25 (q, 2H, *j*= 5.32, CH_2), 7.71-8.23 (m, 7H,Ar-H), 7.62(d, 2H, *j*= 4.24Hz, pyrrole), 7.83 (d, 2H, *j*= 4.24Hz, pyrrole), 8.72,8.90 (2bs, 2H, 2NH, exchangeable with D_2O), 8.52 (s, 1H, pyrimidine); M.S (E.I) m/z% =502(M^+ -H_2S, 20%), 65(100).

4-(4-floro-phenyl)-7-(2-methyl-4-nitro-phenyl)-6-pyrrol-1-yl-2-thioxo-2,3,4,7, tetra- hydro-1H-pyrrolo[2,3-d]pyrimidine-5-carboxylic acid ethyl ester **(11b):**
Crystallized from ethanol
Yield = (72%); mp260-2 ^{o}C., Analyses calculated**:** $C_{26}H_{22}FN_5O_4S$ (519.59); IR (KBr, ν/cm^{-1}): 3402 (NH_2), 1675, (CO); ^{1}H-NMR (DMSO-d_6): δ/ppm =1.78 (t, 3H, *j*=5.40, CH_3), 2.37 (s, 3H, CH_3), 4.25 (q, 2H, *j*= 5.32, CH_2), 7.71-8.23 (m, 7H,Ar-H), 7.52 (d, 2H, *j*= 4.24Hz, pyrrole), 7.93 (d, 2H, *j*= 4.24Hz, pyrrole), 8.72,8.90 (2bs, 2H, 2NH, exchangeable with D_2O), 8.50(s, 1H, pyrimidine); M.S (E.I) m/z% =585(M^+- H_2S, 21%), 486 (M^{+1}-H_2S, 9%), 65 (100).

7-(2-Methyl-4-nitro-phenyl)-4-naphtalen-1-yl-6-pyrrol-1-yl-2-thioxo-2,3,4,7, tetra -hydro-1H-pyrrolo[2,3-d]pyrimidine-5-carboxylic acid ethyl ester **(11c):**
Crystallized from ethanol
Yield = (72%); mp190-2 ^{o}C. , Analyses calculated**:** $C_{30}H_{25}N_5O_4S$ (551.63); IR (KBr, ν/cm^{-1}): 3402 (NH_2), 1675, (CO); ^{1}H-NMR (DMSO-d_6): δ/ppm=1.68(t, 3H, *j*=5.40, CH_3), 2.34 (s, 3H, CH_3), 4.15 (q, 2H, *j*= 5.32, CH_2), 7.51-8.23 (m, 14H, Ar-H, pyrrole), 7.89(s, 1H, pyrimidine), 8.92, 9.01 (2bs, 2H, 2NH, exchangeable with D_2O); M.S (E.I) m/z% =518(M^+ -H_2S, 65%), 98 (100).

Biochemistry

i-Chemicals:
Deltamethrin was synthesized in our laboratory according to known methods [30]

ii –Animals and treatment:
Male albino rats of 10-12 weeks of age weighing 120±20 g were used for the study. The animals housed in polypropylene cages and were fed on pellet diet and water adds libitum. Animals were divided into six groups of six animals in each. Group 1 received dismethylsulfoxide (1mg/kg body weight) orally by using oral feeding needles. Group 2 received deltamethrin at dose of 1/20 LD_{50} (7.5 mg/kg body weight). Group 3 and 5 received compounds **4a** and **9c** (10mg/kg body weight). Group 4 and 6 received each compound in combination with deltamethrin. Treatment duration was once in a day for 30 days. Animals were sacrificed 24 hours after the last treatment. Blood was collected in a two groups of test tubes. The first group kept under refrigeration for separation of serum and the other group was containing heparin as anticoagulant and then centrifuge to separate plasma and blood cells.

iii- Biochemical determination:

Serum was used for determination of alanine and aspartate aminotransferase (ALT and AST), alkaline phosphatase (ALP), catalase (CAT), Glutathione-S-transferase (GST), total protein (TP), albumin (ALB), creatinine, urea, cholesterol (Cho), triglycerides (TG) low density lipoprotein (LDL), and high density lipoprotein (HDL) by using local kits (from Biodiagonostic Company in Egypt) after 30 days of treatment. Heparinized blood was analyzed for cholinesterase enzyme activity according to Ellman methods [31].

Table (1): Change in the activities of cholinesterase enzyme activities of rats treated with deltamethrin (DLM), 4a and 9c compounds for 30 days.

Experimental groups	Plasma-cholinesterase level		Erythrocyte- cholinesterase level	
	Mean ± S.D	Inhibition (%)	Mean ± S.D	Inhibition (%)
Control	2.20 ± 0.17	-	4.12 ± 0.13	-
Deltamethrin	1.68 ± 0.05	24	3.50 ± 0.08	15
4a only	2.30 ± 0.11	-	4.18 ± 0.11	-
DLM +4a	2.04 ± 0.02	7	3.98 ± 0.17	3
9c only	2.35 ± 0.12	-	3.98 ± 0.12	3
DLM +9c	2.12 ± 0.08	4	3.80 ± 0.14	8

Values are expressed as means ± S.D; n = 6 , for each treatment group; Acetyl cholinesterase activity: u mol / min / ml

Table (2): Enzyme activities of rats treated with deltamethrin (DLM), 4a and 9c compounds for 30 days.

Enzyme	Experimental groups					
	Control	**DLM ($1/20LD_{50}$)**	**4a**	**DLM+ 4a**	**9c**	**DLM+ 9c**
ALT (U/L)	34.70±2.47	52.5±2.35*	33.0±1.82	38.0±2.21	34±2.24	34.7±2.00
AST (U/L)	91.30±3.10	123±4.72*	95.0±2.91	93.7±3.64	95±4.50	89.7±3.84
ALP (U/L)	62.80±2.42	80±3.80*	56.5±2.80	63.3±2.93	66 ±2.37	59.0±3.42
Cat (u/mg protein)	0.47 ±0.06	0.72±0.01*	0.45±0.05	0.38±0.03	0.49±0.03	0.41±0.06
GST (μmol/mg protein)	1.44±0.07	0.95±0.06*	1.45±0.09	1.27±0.06	1.45±0.08	1.27±0.04

Values are expressed as means ±SD; n=6 for each treatment group.; Significant at $p<0.05$ in comparison with control.; ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: alkaline phosphatase, Cat: catalase, GST: glutathione S-transferase, AchE: acetyl cholinesterase.

Table (3): Some biochemical parameters in serum of rats treated with deltamethrin(DLM), 4a and 9c compounds for 30 days.

Parameters (g/dl)	Experimental groups					
	Control	DLM (1/20LD_{50})	4a	DLM +4a	9c	DLM 9c+
Total protein	3.79±0.25	2.70±0.23*	3.76±0.16	3.56±0.30	3.86±0.20	3.56±0.18
Albumin	7.43±0.43	5.65±0.28*	7.40±0.37	6.76±0.28	7.95±0.32	6.97±0.46
Urea	40.67±1.8	74.25±2.2*	66.5±2.00	66.7±1.70	50.0±1.50	66.67±1.9
Creatinine	0.697±0.08	1.10±0.09*	0.74±0.04	0.746±0.08	0.745±0.04	0.75±0.06

Data expressed as means ±SD; n=6 for each treatment group.; Significant at $p<0.05$ in comparison with control.

Table (4): Lipid profile and lipoprotein of rats treated with deltamethrin (DLM), 4a and 9c compounds for 30 days.

Lipid (mg/dl)	Control	DLM (1/20LD_{50})	4a	DLM+ 4a	9c	DLM+ 9c
cholesterol	103±4.7	123±6.20*	90.5±5.0	84.3±4.8	102.5±5.1	93.3±4.3
Triglycerides	84.7±4.0	135.5±7.4*	86.0±4.6	82.7±3.9	82.5±3.8	77.6±2.5
HDL	22.3±2.6	14.5±1.90*	19.5±2.3	17.3±1.6	20.5±1.7	18.7±2.0
LDL	64.1±3.7	80.9±4.00*	53.8±2.1	50.5±3.3	65.0±3.8	59.2±2.6

Values are expressed as means ±SD; n=6 for each treatment group.; Significant at $p<0.05$ in comparison with control.; HDL: high density lipoprotein, LDL: low density lipoprotein.

Conclusion

We concluded that the use of *ANRORC* approach is a valuable method for the obtainment of ring transformation of heterocyclic systems**.** Therefore, we described several efficient approaches to heteroaromatic systems using functionally substituted enamine precursors.

The present study revealed that compounds **4a** and **9c** alleviated the harmful effect of deltamethrin on rats and reduced its adverse effects on most biochemical parameters.

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