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Synthesis, Characterization and Biological Evaluation of Some New Oxino *bis*-Pyrazole Derivatives

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Novel oxino *bis*-pyrazoles were obtained by two step reactions of 3-methyl-1-phenyl pyrazoline-5-one, cyanoacetamide and aryl- or alkylaldehydes, in the presence of catalytic amounts of triethylamine and ethanol as a solvent. All the synthesized compounds were evaluated for their inhibitory activities against butyrylcholinesterase (BChE) and bovine α-chymotrypsin. Two compounds, **8** and **9** were found to be good inhibitors of enzyme BChE with IC_{50} values of 52.74 ± 0.006 and 51.85 ± 0.005 μM, respectively.

Keywords: *Bis*-pyrazoles, Multicomponent reactions, Butyrylcholinesterase (BChE), Bovine α-Chymotrypsin.

INTRODUCTION

Pyrazoles are very important class of biologically active compounds and many of their derivatives are in clinical use. Pyrazole derivatives have been shown to exhibit antihyperglycemic [1], hypoglycemic [2] and fungicidal properties [3]. Some of them are also used as insecticides and herbicides [4,5]. Of the condensed pyrazoles, specially pyrano[2,3-*c*]pyrazole is a fused heterocycle comprising of pyrazole and pyran rings which are known as the sub-structural units of several biologically active compounds [6,7]. While structurally similar polyfunctionalized benzopyrans have been widely used as intermediates due to their biological and pharmacological properties such as antibacterial, molluscicidal, anthelminitic, hypnotic and insecticidal activity [8-14]. Some 2-amino-4*H*-pyrans are used as photoactive materials [15] where as the 4*H*-pyran ring is also a structural unit of a number of natural products [16-18].

EXPERIMENTAL

All commercial reagents and solvents, unless specified, were used as received without further purification. The reactions were monitored and R_f value were determined using analytical thin layer chromatography (TLC) with Merck Silica gel 60 and F-254 precoated plates (0.25 mm thickness). Spots on the TLC plates were visualized using ultraviolet light (254 nm). Flash column chromatography was performed with Merck silica gel 60 (100-200 mesh). Melting points were determined in capillaries and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 400 and 500 NMR spectrometers and infrared spectra on a Perkin Elmer FT-IR 400 spectrometer. Mass spectral analyses were carried out on Agilent Technologies 1100 Series instrument.

General procedure for the preparation of *bis*-pyrazoles: To a stirred ethanolic mixture of cyanoacetamide (0.005 mol, 0.42 g) and the respective aldehydes (0.005 mol), few drops of triethylamine were added successively at room temperature with vigorous stirring for 3 min to overnight. The solid (Z)-2-cyano-3-alkyl or arylacrylamide (**1**) thrown out from the reaction mixture was further reacted with 3-methyl-1-phenylpyrazoline-5-one (0.005 mol, 0.87 g) (**2**) to give a *bis*-pyrazole derivative **4** (**Scheme-I**). The crude products were purified by washing with ethanol. The products obtained were also found to be pure on TLC and NMR spectra.

Following products were obtained from various reactions:

3,5-Dimethyl-1,4,7-triphenyl-8-oxa-1,2,6,7-tetraza-4,7-dihydro-1*H*-s-indacene (5): Yield: 2.52 g (98 %) white crystals, m.p. 142-144 °C. IR (KBr, ν_{max}, cm^{-1}): 1593, 1577, 1491, 1410, 1026, 792, 759, 688, 675. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.09 (s, 6H, C<u>H</u>$_3$), 4.77 (s, 1H, Ph-C<u>H</u>), 7.11 (t, 2H, Ph<u>H</u>, *J* = 7.4 Hz), 7.23 (m, 9H, Ph<u>H</u>), 7.53 (d, 2H, Ph<u>H</u>, *J* = 8.9 Hz), 7.55 (d, 2H, Ph<u>H</u>, *J* = 7.7 Hz). ^{13}C NMR (125 MHz, $CDCl_3$): δ =11.53, 18.38, 30.92, 33.68, 58.37, 105.70,

121.36, 126.20, 126.41, 127.17, 128.34, 128.85, 136.96, 140.90, 146.41, 157.66. EIMS, *m/z* (%): 418.2 (not observed), 406.2 (5), 384.3 (8), 287.1 (10), 262.1 (95), 185.1 (97), 174.1 (44), 128.1 (2), 105.1 (38), 91.1 (68), 77.1 (79), 67.1 (40).

4-(2-Furyl)-3,5-dimethyl-1,7-diphenyl-8-oxa-1,2,6,7-tetraza-4,7-dihydro-1*H*-*s*-indacene (6): Yield: 1.0 g (49 %), m.p. 182-183 °C. IR (KBr, ν_{max}, cm^{-1}): 1595, 1577, 1496, 1408, 1373, 1282, 1008, 806, 785, 763, 752, 731, 673. ^{1}H NMR (400 MHz, DMSO-d_6): δ =2.30 (s, 6H, C<u>H</u>$_3$), 4.98 (s, 1H, Ar-C<u>H</u>), 6.12 (dd, 1H, Furan, *J* = 2.14, 1.07 Hz), 6.35 (dd, 1H, Furan, *J* = 1.30, 1.86 Hz), 7.25 (t, 2H, Ph<u>H</u>, *J* = 7.31 Hz), 7.45 (t, 4H, Ph<u>H</u>, *J* = 8.20 Hz) 7.51 (s, 1H, Furan), 7.72 (d, 4H, PhH, *J* = 7.66 Hz). ^{13}C NMR (125 MHz, MeOD): δ =10.20, 28.56, 47.09, 48.11, 106.62, 121.73, 126.56, 127.03, 128.69, 129.10, 130.21, 141.17, 144.48, 154.12.

3,5-Dimethyl-1,7-diphenyl-4-(2-thienyl)-8-oxa-1,2,6,7-tetraza-4,7-dihydro-1*H*-*s*-indacene (7): Yield: 1.82 g (85 %), m.p. 194-195 °C. IR (KBr, ν_{max}, cm^{-1}): 1595, 1575, 1492, 1408, 1292, 931, 914, 783, 758, 669. ^{1}H NMR (400 MHz, DMSO-d_6): δ =2.34 (s, 6H, C<u>H</u>$_3$), 5.15 (s, 1H, Ar-C<u>H</u>), 6.77 (t, 1H, Thiophene, *J* = 1.94 Hz), 6.92 (dd, 1H, Thiophene, *J* = 5.06, 3.56 Hz), 7.26 (t, 2H, Ph<u>H</u>, *J* = 7.34 Hz), 7.31 (d, 1H, Thiophene, *J* = 5.04 Hz), 7.46 (t, 4H, Ph<u>H</u>, *J* = 8.12 Hz), 7.74 (d, 4H, Ph<u>H</u>, *J* = 7.71 Hz). ^{13}C NMR (125 MHz, DMSO-d_6): δ =11.96, 29.93, 40.13, 121.06, 124.62, 126.11, 127.24, 129.41, 146.32, 147.90, 156.40, 157.09, 158.23.

3,5-Dimethyl-1,7-diphenyl-4-(2-pyridyl)-8-oxa-1,2,6,7-tetraza-4,7-dihydro-1*H*-*s*-indacene (8): Yield: 1.0 g (67 %), m.p. 193-195 °C. IR (KBr, ν_{max}, cm^{-1}): 1593, 1518, 1489, 1410, 1352, 1273, 1099, 1031, 1008, 896, 785, 690, 678. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.29 (s, 6H, C<u>H</u>$_3$), 5.12 (s, 1H, Ar-C<u>H</u>), 7.24 (m, 3H, Ar<u>H</u>), 7.44 (m, 5H, Ar<u>H</u>), 7.72 (d, 2H, Ar<u>H</u>, *J* = 7.82 Hz), 7.79 (dd, 3H, Ar<u>H</u>, *J* = 6.58, 0.93 Hz), 8.51 (d, 1H, Ar<u>H</u>, *J* = 4.06 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 12.11, 37.05, 114.60, 120.81, 122.13, 122.44, 125.82, 129.34, 137.68, 138.20, 147.10, 148.64, 157.32, 161.72. HRMS, *m/z* (%): 460.2 (M^+K) (100), 482.2 (47), 501.2 (32), 523.2 (31).

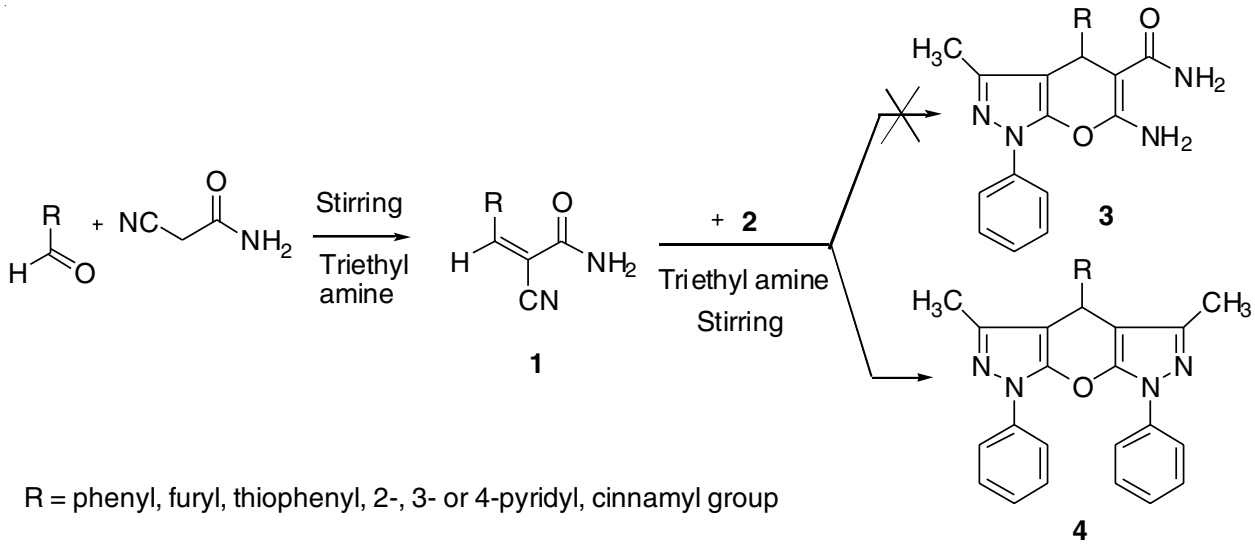
3,5-Dimethyl-1,7-diphenyl-4-(3-pyridyl)-8-oxa-1,2,6,7-tetraza-4,7-dihydro-1*H*-*s*-indacene (9): Yield: 2.60 g (98 %), m.p. 248-249 °C. IR (KBr, ν_{max}, cm^{-1}): 1593, 1577, 1496, 1421, 1354, 1290, 1049, 1028, 798, 746, 694. ^{1}H NMR (400 MHz, DMSO-d_6): δ = 2.35 (s, 6H, C<u>H</u>$_3$), 5.06 (s, 1H, Ar-C<u>H</u>), 7.25 (t, 2H, Ph<u>H</u>, *J* = 7.38 Hz), 7.35 (m, 1H, Ar<u>H</u>), 7.44 (m, 4H, Ar<u>H</u>), 7.73 (dd, 5H, Ar<u>H</u>, *J* = 8.48, 0.88 Hz), 8.43 (dd, 1H, Ar<u>H</u>, *J* = 4.78, 1.18 Hz), 8.51 (d, 1H, Ar<u>H</u>, *J* = 2.27 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ = 12.11, 31.55, 104.50, 121.09, 123.84, 126.03, 129.21, 129.40, 125.78, 138.45, 146.70, 147.23, 149.00, 157.42.

3,5-Dimethyl-1,7-diphenyl-4-(4-pyridyl)-8-oxa-1,2,6,7-tetraza-4,7-dihydro-1*H*-*s*-indacene (10): Yield 1.90 g (91 %), m.p. 160-162 °C. IR (KBr, ν_{max}, cm^{-1}): 1699, 1593, 1575, 1494, 1489, 1410, 1276, 1066, 1024, 688, 678. ^{1}H NMR ($CDCl_3$): δ = 2.06 (s, 6H, C<u>H</u>$_3$), 4.60 (s, 1H, Ar-C<u>H</u>), 6.99 (t, 2H, Ph<u>H</u>, *J* = 7.42 Hz), 7.07 (d, 2H, Ar<u>H</u>, *J* = 5.76 Hz), 7.13 (t, 4H, Ph<u>H</u>, *J* = 7.74 Hz), 7.47 (d, 4H, Ph<u>H</u>, *J* = 7.76 Hz), 8.12 (d, 2H, Ar<u>H</u>, *J* = 6.09 Hz). ^{13}C NMR ($CDCl_3$): δ = 8.34, 11.80, 15.24, 33.63, 45.60, 65.82, 121.41, 123.26, 126.25, 128.93, 137.19, 146.16, 148.57. HRMS, *m/z* (%): 460.2 (M^+K) (23), 482.2 (20), 501.2 (24), 523.2 (100).

(*E*)-2-(3,5-Dimethyl-1,7-diphenyl-8-oxa-1,2,6,7-tetraza-4,7-dihydro-1*H*-*s*-indacen-4-yl)-1-phenyl-1-propene (11): Yield: 1.75 g (76 %), m.p. 178-180 °C. IR (KBr, ν_{max}, cm^{-1}): 1737, 1597, 1577, 1502, 1375, 1348, 1332, 1255, 1232, 1186, 1022, 852, 750, 613. ^{1}H NMR (400 MHz, $CDCl_3$) δ =1.52 (s, 3H, C<u>H</u>$_3$), 1.87 (s, 6H, C<u>H</u>$_3$), 3.90 (s, 1H, Ar-C<u>H</u>), 6.16 (s, 1H, Ph-C<u>H</u>), 6.89 (t, 6H, Ph<u>H</u>, *J* = 7.41 Hz), 7.09 (t, 3H, Ph<u>H</u>, *J* = 5.96 Hz) 7.42 (d, 4H, PhH, *J* = 7.66 Hz). ^{13}C NMR (125 MHz, MeOD): δ =10.31, 16.50, 24.43, 37.32, 114.70, 121.61, 125.30, 125.68, 126.48, 127.60, 128.64, 136.10, 138.50, 155.76, 156.32, 156.85.

RESULTS AND DISCUSSION

Since various reactions of compound **2** with malononitrile and arylaldehydes have been reported to give 6-amino-4-aryl-5-cyano-3-methyl-1-phenyl pyrano[3,4,c]pyrazoles [19], it was expected that a similar reaction with cyanoacetamide will provide the expected product **3** (**Scheme-I**). However a close examination of the analytical data from a reaction with benzaldehyde showed absence of any absorption due to an expected amino functional group and a carbonyl of an amide



Scheme-I: Synthesis of *bis*-pyrazoles **4** with alkyl and aryl aldehydes

group in its IR spectra. The ^{1}H NMR spectra also corroborated by complete absence of any such signals but instead a simpler spectrum which seems to be a symmetrical structure showing one signal for six methyl protons at δ = 2.09, fifteen aromatic protons in the region δ = 7.11-7.55 and the one key proton of Ar-CH at δ = 4.77. This data fits in well with the proposed structured **4** (**Scheme-I**).

When the reaction was repeated with other aldehydes, again the tricyclic products (**5-11**) (Fig. 1, Table-1) were obtained without any trace of the corresponding **3**. All the products had the supporting spectral data. Compounds **5-11** also showed an absence of an amide C=O carbon in its ^{13}C NMR spectrum.

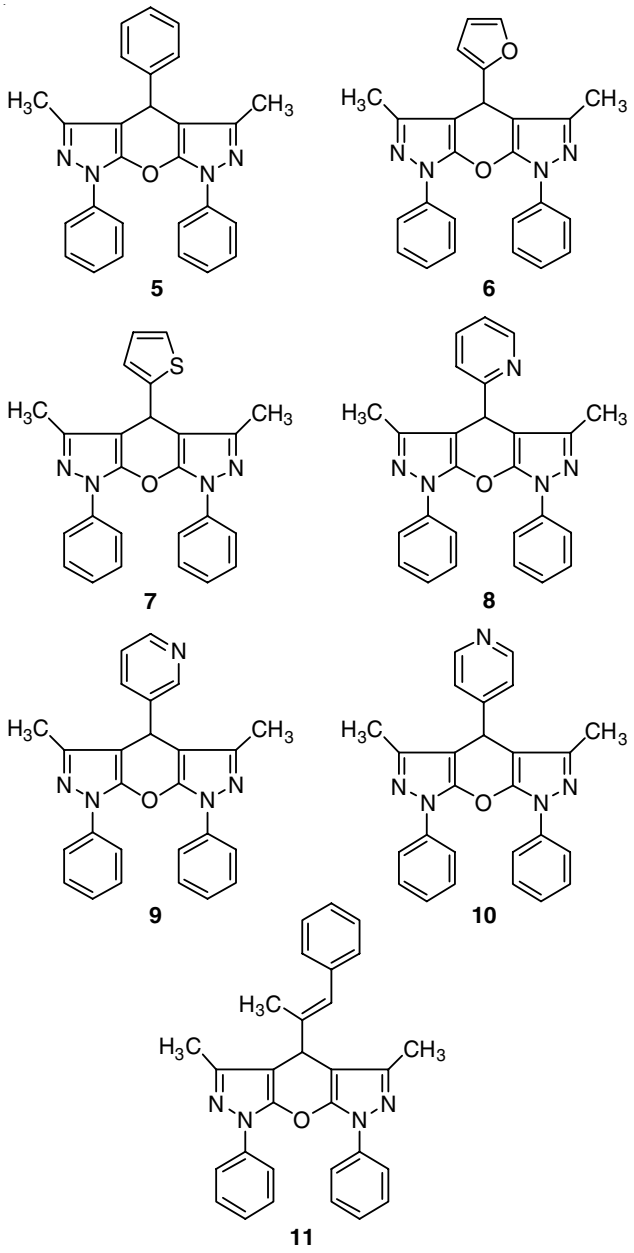


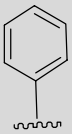
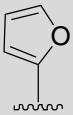
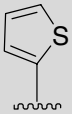
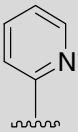
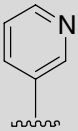
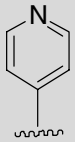
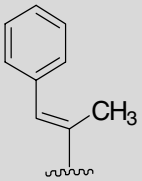
Fig. 1. Structures of some selected *bis*-pyrazoles

A following possible mechanism accounts for the reaction: one molecule of an arylaldehyde firstly condenses with cyanoacetamide to afford a (Z)-2-cyano-3-arylacrylamide derivative **1**. The adduct **1** then reacts with 3-methyl-1-phenyl-2-pyrazoline-5-one (**2**), *via* the initial Michael addition to afford an "acyclic Michael adduct" which then loses the active methylene moiety, *i.e.*, cyanoacetamide to give the arylidene pyrazolone which is attacked by a new molecule of **2** followed by a cyclodehydration step to yield the isolated *bis*-pyrazole derivative **4** (**Scheme-II**).

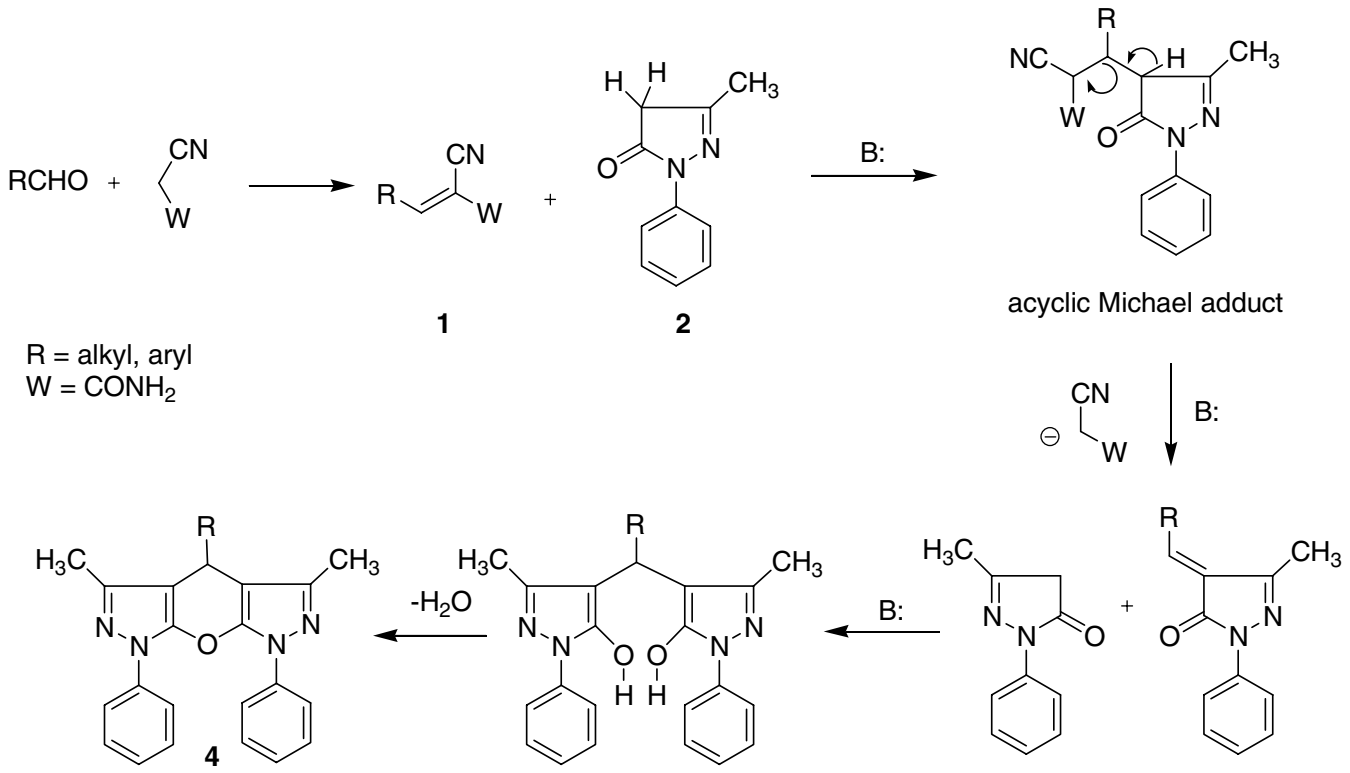
This product formation has a parallel with a similar reaction of ethyl cyanoacetate and 3-methyl-1-phenyl-2-pyrazoline-5-one with aldehydes. Here also no 2-amino-3-carbothoxy compound was isolated or observed, again a tricyclic product was formed [20].

It appears that the reaction is independent of the nature of aldehydes whether they are aromatic or heteroaromatic (π excessive or π deficient). All these seem to have reacted well to give the corresponding **5-11** in good to excellent yields (Table-1). Some of these reactions are very fast for example, 3,5-dimethyl-1,7-diphenyl-4-(3-pyridyl)-8-oxa-1,2,6,7-tetraza-4,7-dihydro-1*H*-*s*-indacene (**9**) crashing out within 3 min with a 98 % yield.

TABLE-1
SYNTHESIS OF *BIS*-PYRAZOLES
FROM DIFFERENT ALDEHYDES

Product No.	R* groups	Time	Yield (%)	m.p. (°C)
5		1 h	98	142-144
6		20 min	49	182-183
7		20 min	85	194-195
8		4 h	67	193-195
9		3 min	98	248-249
10		4 h	91	160-162
11		Over night	76	178-180

* See **Scheme-I**



Scheme-II: Proposed mechanism

Biological activity: All the prepared compounds (**5-11**) were screened for their activity against butyrylcholinesterase (BChE) and bovine α-chymotrypsin (Table-2) [21]. The pyridyl substitttuted compounds (**8** and **9**) were found to be relatively good inhibitors of BChE with IC_{50} values of 52.74 ± 0.006 and 51.85 ± 0.005 μM, respectively when compared to that of standard inhibitor eserine with IC_{50} value 0.85.79 ± 0.0001 μM.

TABLE-2
BChE AND BOVINE α-CHYMOTRYPSIN ACTIVITY OF THE COMPOUNDS

Test compound	Butyrylcholinesterase		α-Chymotrypsin	
	Inhibition (%) at 0.5 mM	IC_{50} (μM)	Inhibition (%) at 0.5 mM	IC_{50} (μM)
5	75.36±0.64	118.82±0.25	31.84±1.12	–
6	26.46±0.12	–	47.23±0.67	–
7	39.84±0.17	–	43.61±0.85	–
8	89.77±0.12	52.74±0.006	43.23±0.78	–
9	89.56±0.15	51.85±0.005	51.15±0.93	–
10	78.43±0.11	98.79±0.06	33.74±0.87	–
11	86.26±0.14	84.52±0.08	21.38±0.67	–
Eserine	82.82±1.09	0.85.79±0.0001	–	–
PMSF (standard)	–	–	96.71±0.79	48.71± 0.13

All compounds were prepared in DMSO. All the measurements were done in triplicate. Results are presented as mean ± SEM.

On the other hand, all the compounds **5-11** exhibited low inhibition profile against α-chymotrypsin at 0.5 μM tested concentration.

Conclusion

Some novel oxino *bis*-pyrazoles (**7-11**) were isolated from the reaction of 3-methyl-1-phenyl-2-pyrazoline-5-one, aryl-aldehydes and cyanocetamide. Their structure was elucidated from their spectral data. The reaction has been shown to display relatively good symmetry in structure, excellent yields and product isolation is very straight forward. A possible mechanism for their formation is forwarded. These novel compounds showed good activity against BChE.

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