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Synthesis and Evaluation of Antioxidant Properties of N-substituted α-cyanocinnamides

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Abstract. Derivatives of N-substituted α-cyanocinnamides were synthesized and evaluated for anti-oxidant properties by*in vitro* models like reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Scavenging of Nitric Oxide (NO). Several compounds had good to moderate anti-oxidant activity. Compounds containing N-4-hydroxyphenyl moietyand substituted with 3,5-dimethoxy-4-hydroxy group on α-cyanocinnamide (**3h**) exhibited excellent *in vitro* anti-oxidant activity.

Keywords: α-cyanocinnamides, knoevenagel condensation, anti-oxidant properties.

1. Introduction

N-substituted α-cyanocinnamides possess diverse biological activities such as antitumor (Wei Zhou et al., 2009), antiparkinson's, antiplatelet (Chia-Cheng Hung et al., 2005), anti-inflammatory (IkuoKastumi, et al., 1986) activities etc., In view of potential biological activities of N-substituted α-cyanocinnamides against diseases associated with oxidative stress, it has been considered worthwhile to synthesize new N-substituted α-cyanocinnamides and to evaluate themfor their *in vitro* anti-oxidant properties.Further, asimple method (Nikolay et al., 2004) has been used for synthesizing N-substituted cyanoacetamides as starting materials. The title compounds were synthesized byknoevenagel condensation of N-substituted cyanoacetamide and substituted benzaldehydes as shown in scheme-1.

2. Experimental

The melting points reported were determined in open capillaries, using Stuart melting point apparatus and are uncorrected. TLC was performed using glass plates coated with silica gel G and the spots were detectedby iodine vapour. IR spectra (KBr, $\upsilon_{max,}cm^{-1}$) were run on Perkin Elmer FTIR Spectrophotometer. ^{1}H NMR spectra (chemical Shifts in δ ppm) were recorded on Avance-300 MHz Spectrometer using TMS as internal standard and mass spectra were recorded on Shimadzu QP 2010PLUS GC-MS system. All the chemicals were obtained from Merck, Sd-fine, Aldrich and Sigma of AR grade.

2.1 Chemistry

2.1.1 General procedure for the synthesis of N-Substituted α-cyanocinnamide (3a-3l)

To a solution of N-substituted α-cyanoacetamide (10mmol) in toluene (50 ml), 10mmol of substituted benzaldehyde was added. To this mixture, 0.35ml piperidine and 1.3ml of acetic acid was added and refluxed for 5-6 hours. The completion of the reaction was monitored by performing TLC. Then the reaction mixture was cooled to room temperature, the precipitate was separated by filtration. The product washed and recrystallized with suitable solvent.

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2.1.1.1 2-cyano-N,3-diphenylacrylamide (3a): IR 3340 cm^{-1} (N-H), 3055 cm^{-1} (Ar C-H), 2214 cm^{-1} (C≡N), 1681 cm^{-1} (C=O);^{1}H NMR δ 7.1-8.0 (m, 10H, Ar), δ 8.35 (s, 1H, -CH=), δ 10.4 (s,1H, NH) ppm; Mass m/z 248 (M^+).

2.1.1.2 2-cyano-3-(4-hydroxyphenyl)-N-phenylacrylamide (3b): IR 3329 cm^{-1} (N-H), 3206 (O-H), 3151 cm^{-1} (Ar C-H), 2350 cm^{-1} (C≡N), 1659 cm^{-1} (C=O);^{1}H NMR δ 6.9-7.9 (m, 9H, Ar), δ 8.1 (s, 1H, -CH=), δ 10.2 (O-H), δ 10.4 (s,1H, NH) ppm; Mass m/z 264 (M^+).

2.1.1.3 2-cyano-3-(4-hydroxy-3-methoxyphenyl)-N-phenylacrylamide (3c): IR 3291 cm^{-1} (N-H & O-H), 3063 cm^{-1} (Ar C-H), 2280 cm^{-1} (C≡N), 1671 cm^{-1} (C=O);^{1}H NMR δ 3.9 (s, 3H, OCH_3), δ 7.0-7.7 (m, 9H, Ar), δ 8.2 (s, 1H, -CH=), δ 10.2 (s,1H, NH) ppm; Mass m/z 294 (M^+).

2.1.1.4 2-cyano-3-(4-hydroxy-3,5-dimethoxyphenyl)-N-phenylacrylamide (3d): IR 3298 cm^{-1} (N-H & O-H), 3055 cm^{-1} (Ar C-H), 2209 cm^{-1} (C≡N), 1659 cm^{-1} (C=O);^{1}H NMR δ 4.0 (s, 6H, OCH_3), δ 7.2-7.6 (m, 7H, Ar), δ 8.0 (s, 1H, -CH=), δ 8.3 (s,1H, NH) ppm; Mass m/z 323 (M^+-1).

2.1.1.5 2-cyano-N-(4-hydroxyphenyl)-3-phenylacrylamide (3e): IR 3341 cm^{-1} (N-H), 3240 cm^{-1} (O-H), 3081cm^{-1} (Ar C-H), 2219 cm^{-1} (C≡N), 1652 cm^{-1} (C=O);^{1}H NMR δ 6.8-7.9 (m, 10H, Ar), δ 8.24 (s, 1H, -CH=), δ 9.35 (s, 1H,OH), δ 10.1 (s,1H, NH) ppm; Mass m/z 264 (M^+).

2.1.1.6 2-cyano-N,3-bis(4-hydroxyphenyl)acrylamide (3f): IR 3314 cm^{-1} (N-H), 3225 cm^{-1} (O-H), 3055 cm^{-1} (Ar C-H), 2315 cm^{-1} (C≡N), 1660 cm^{-1} (C=O);^{1}H NMR δ 6.7-7.9 (m, 9, Ar), δ 8.1 (s, 1H, -CH=), δ10.0 (s,1H, NH) ppm; Mass m/z 280 (M^+).

2.1.1.7 2-cyano-3-(4-hydroxy-3-methoxyphenyl)-N-phenylacrylamide (3g): IR 3372 cm^{-1} (N-H), 3522 cm^{-1} (O-H), 3081cm^{-1} (Ar C-H), 2342 cm^{-1} (C≡N), 1645 cm^{-1} (C=O);^{1}H NMR δ 3.8 (s, 3H, OCH_3), δ 6.7-7.7 (m, 7H, Ar), δ 8.1 (s, 1H, -CH=), δ 9.3 (s, 1H,OH), δ 10.0 (s,1H, NH), 10.3 (s, 1H, OH) ppm; Mass m/z 310 (M^+).

2.1.1.8 2-cyano-3-(4-hydroxy-3,5-dimethoxyphenyl)-N-phenylacrylamide (3h): IR 3341 cm^{-1} (N-H), 3240 cm^{-1} (O-H), 3081cm^{-1} (Ar C-H), 2219 cm^{-1} (C≡N), 1652 cm^{-1} (C=O);^{1}H NMR δ 3.8-4.0 (2s, 6H, OCH_3), δ 5.8 (s, 1H, OH), δ 7.1-7.49 (m, 6H, Ar), δ 8.2 (s, 1H, -CH=), δ 9.1 (s, 1H,OH), δ 9.8 (s,1H, NH) ppm; Mass m/z 339 (M^+-1).

2.1.1.9 2-cyano-N-(5-methylisoxazol-3-yl)-3-phenylacrylamide (3i): IR 3221 cm^{-1} (N-H), 3085cm^{-1} (Ar C-H), 2223 cm^{-1} (C≡N), 1676 cm^{-1} (C=O);^{1}H NMR δ 2.5 (s, 1H, CH_3), δ 6.74 (s, 1H, isoxazolyl), δ 7.5-8.03 (m, 5H, Ar), δ 8.43 (s, 1H, -CH=), δ 8.7 (s,1H, NH) ppm;Mass m/z 254 (M^+).

2.1.1.10 2-cyano-3-(4-hydroxyphenyl)-N-(5-methylisoxazol-3-yl)-acrylamide (3j): IR 3352 cm^{-1} (O-H), 3256 cm^{-1} (N-H), 3083cm^{-1} (Ar C-H), 2228 cm^{-1} (C≡N), 1678 cm^{-1} (C=O); Mass m/z 269 (M^+).

2.1.1.11 2-cyano-3-(4-hydroxy-3-methoxyphenyl)-N-(5-methylisoxazol-3-yl)-acrylamide (3k): IR 3366 cm^{-1} (O-H), 3279 cm^{-1} (N-H), 3082cm^{-1} (Ar C-H), 2215 cm^{-1} (C≡N), 1687 cm^{-1} (C=O);^{1}H NMR δ 2.45 (s, 1H, CH_3), δ 3.9 (s, 3H, OCH_3), δ 6.74 (s, 1H, isoxazolyl), δ 7.1-8.0 (m, 10H, Ar), δ 8.35 (s, 1H, -CH=), δ 10.4 (s,1H, NH) ppm;Mass m/z 300 (M^+).

2.1.1.12 2-cyano-3-(4-hydroxy-3,5-dimethoxyphenyl)-N-(5-methylisoxazol-3-yl)-acrylamide (3l); IR 3317 cm^{-1} (N-H), 3258 cm^{-1} (O-H), 3093cm^{-1} (Ar C-H), 2214 cm^{-1} (C≡N), 1670 cm^{-1} (C=O)

2.1.2 In Vitro Anti-oxidant activity

The title compounds were evaluated for *invitro* anti-oxidant activity by assay of DPPH (1,1-diphenyl -2-picryl hydrazyl) scavenging(Blois MS, 1958) and assay of NO (Nitric oxide) scavenging (Sreejayan&Rao MNA, 1997).

Assay of DPPH radical scavenging

The test compounds at 100 µM concentrations were added to 100 µMDPPH in 95% ethanol. The test tubes were kept at ambient temperature for 20 minutes and the absorbances were measured at 517 nm. Control experiment was carried out with solvent only. All the measurements were run in triplicate. The percentage of scavenging activity was calculated as follows:

Percentage Scavenging = [Control-Test]/Control × 100

Assay of NO radical scavenging

Sodium nitroprusside (10 μM) in phosphate buffer pH 7.4 was incubated with 100 μM concentration of drug dissolved in alcohol and tubes were incubated at 25□ C for 150 minutes. Control experiment was kept without test compound but an equal amount of solvent was added in an identical manner. 2 ml of incubation solution was removed and diluted with 2ml of griess reagent. The absorbance of the chromophore formed during diazotization of nitrile with sulphanilamide and subsequent naphthylethylenediamine was read at 546 nm. All the measurements were runin triplicate and the percentage of scavenging activity was calculated as above formula.

3. Results and Discussion

In the present study, the N-substituted α-cyanocinnamides were synthesized from three different N-substituted cyanoacetamidesnamely, N-phenyl cyanoacetamide (**1a**), N-(4-hydroxyphenyl)cyanoacetamide (**1b**) and N-(5-methylisoxazol-3-yl)cyanoacetamide (**1c**) as starting materials and they were synthesized based on available literature. These compounds (**1a-1c**) upon reaction with various substituted benzaldehydes in toluene with catalytic amounts of piperidine and acetic acid resulted in the formation of N-substituted α-cyanocinnamide derivatives (**3a-3l**). The progress of reaction was monitored by TLC and was completed within 5-6 hours. The percentage yields and melting points of the synthesized compounds were presented in Table-I. The structures of these compounds were confirmed by IR, ^{1}H NMR and Mass spectra.

All the synthesized compounds were evaluated for antioxidant properties by *in vitro* methodsviz DPPH free radical scavenging and Nitric oxide free radical scavenging andresults were summarized in Table-I.The series of N-substituted α-cyanocinnamide derivatives, compounds with N-4-hydroxyphenyl moiety (**3e-3h**) showed good anti-oxidant properties in both the methods, whereas compounds with simple N-phenyl moiety (**3a-3d**) exhibited lower activity. The activity of compounds **3e-3h**was found to be greater than the standard α-tocopherol. This observation reveals that introduction of phenolic hydroxyl group on N-phenyl moiety was necessary for better activity. Replacement of N-phenyl moiety with N-5-methylisoxazole moiety, as in compounds **3i-3l**, did not improve the activity. The activity data of the compounds revealed that, compounds containing 3,5-dimethoxy-4-hydroxy and 4-hydroxy-3-methoxy substitution on α-cyanocinnamide (**3h** and **3g** respectively) greatly enhances the activity.

4. Conclusion

In conclusion, a set of N-substituted α-cyanocinnamides containing N-phenyl, N-4-hydroxyphenyl and N-5-methylisoxazole moieties were prepared by a simple synthetic method form the respective N-substituted α-cyanoacetamides. Compounds **3g** and **3h** exhibited highest activity than standard α-tocopherol.Hence, they may further be evaluated for diseases associated with oxidative stress such as cancer, inflammation, Parkinsonism, Alzheimer's disease.

5. Acknowledgements

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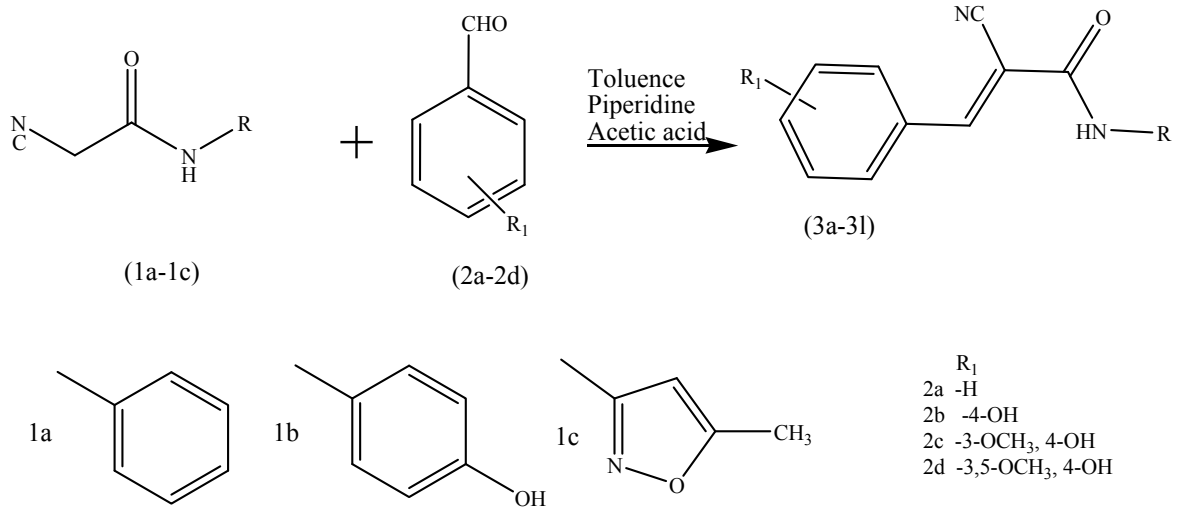

Scheme-1

Table-I

Compound	R_1	Mol. Formula	M.P (^{0}C)	Yield (%)	% Scavenging	
					DPPH	NO
3a	H	$C_{16}H_{12}N_2O$	202-204	76	18.10	08.94
3b	4-OH	$C_{16}H_{12}N_2O_2$	183-185	84	12.40	22.80
3c	3-OCH_3,4-OH	$C_{17}H_{14}N_2O_3$	161-164	67	31.81	27.50
3d	3,5 $(OCH_3)_2$, 4-OH	$C_{18}H_{16}N_2O_4$	172-175	58	30.83	26.31
3e	H	$C_{16}H_{12}N_2O_2$	239-241	68	61.26	35.11
3f	4-OH	$C_{16}H_{12}N_2O_3$	231-236	72	72.15	53.20
3g	3-OCH_3,4-OH	$C_{17}H_{14}N_2O_4$	225-228	67	72.73	66.34
3h	3,5 $(OCH_3)_2$, 4-OH	$C_{18}H_{16}N_2O_5$	196-200	61	75.22	67.48
3i	H	$C_{14}H_{11}N_3O_2$	179-181	61	15.17	13.42
3j	4-OH	$C_{14}H_{11}N_3O_3$	248-252	91	10.76	19.30
3k	3-OCH_3,4-OH	$C_{15}H_{13}N_3O_4$	198-200	78	25.45	26.10
3l	3,5 $(OCH_3)_2$, 4-OH	$C_{16}H_{15}N_3O_5$	216-220	81	30.99	28.29
*	α-tocopherol	-	-	-	61.67	58.74