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Monoamine oxidase-A inhibitory activity of novel Curcumin analogues

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Abstract: Curcumin has been known for its antidepressant activity, but its use has been restricted due to its poor pharmacokinetic properties. In an effort to enhance its permeability and metabolic stability, a series of four novel curcumin analogues (**5a-5d**) were synthesized. They were tested for their hMAO inhibitory activity as well as for their permeability and metabolic stability characteristics in *in vitro* models. The newly synthesized compounds were found to be potent when compared with curcumin and also they are selective and reversible towards hMAO-A. Compounds **5c** and **5d** were found to be potent inhibitors of hMAO-A with *Ki values of Ki*=0.11±0.01*μM* and *Ki*=0.06±0.002 *μM* and with selectivity index of $SI_{MAO\text{-}A}$=802.36 and $SI_{MAO\text{-}A}$=507.33, respectively. A slight enhancement in metabolic stability is achieved through the chemical modification.

Keywords: Curcumin analogue; hMAO inhibitors; Caco-2 permeability; Human liver microsomal metabolic stability.

1. Introduction

Curcumin, is a natural polyphenolic compound obtained from *curcuma longa*, its therapeutic utilities have been well documented in reviews by many researchers.[1-6] Xu *et al.* was the first to report antidepressant activity of curcumin in mice and rat models in 2005.[7,8] Subsequently (in the year 2008), they were also successful in establishing the effect of curcumin on serotonergic system.[9] In the same year, Kulkarni *et al.* reported the increased level of serotonin following the administration of curcumin due to its inhibitory effect on MAO-A isoform.[10] Further the effect of curcumin on MAO isoforms and on animal models for depression were reported by few researchers.[11-18] But due to its poor oral bioavailability,[19,20] blood brain barrier permeability[21] and extensive metabolism in liver,[22,23] curcumin has never been recognized as a drug candidate. Literature review suggests very few attempts made to improve its bioavailability through novel delivery system.[24-28] Few reports are also available on synthetically modified curcumin and are largely explored for their anticancer activity.[29-33]

The current study explores the design of novel curcumin analogues (**Figure 1**) and their inhibitory activity on hMAO isoforms.

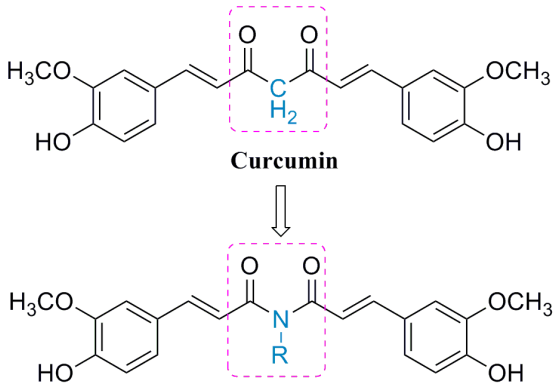

Figure 1. Designed strategy adopted for novel curcumin analogues (**5a-5d**)

2. Result and discussion

2.1. Synthesis

Curcumin analogues (**5a-5e**) were synthesized according to the reactions outlined in **Scheme 1**. Ferulic acid (**1**) was prepared by the reaction of vanillin with malonic acid, in the presence of pyridine and aniline in toluene.[34] The hydroxyl group of ferulic acid (**1**) was protected using acetic anhydride and DMAP to provide acetylated ferulic acid (**2**).[35] Chlorination of acetylated ferulic acid (**2**) was achieved by oxalyl chloride with catalytic amount of DMF in dry dichloromethane at 0-10 °C. That has provided acetylated feruloyl chloride (**3**).[36] Further amidation reactions were processed immediately without any delay because acetylated feruloyl chloride (**3**) is very unstable and provided poor yield upon any delayed processing. Intermediate **4a** was synthesized by refluxing acetylated feruloyl chloride(**3**)

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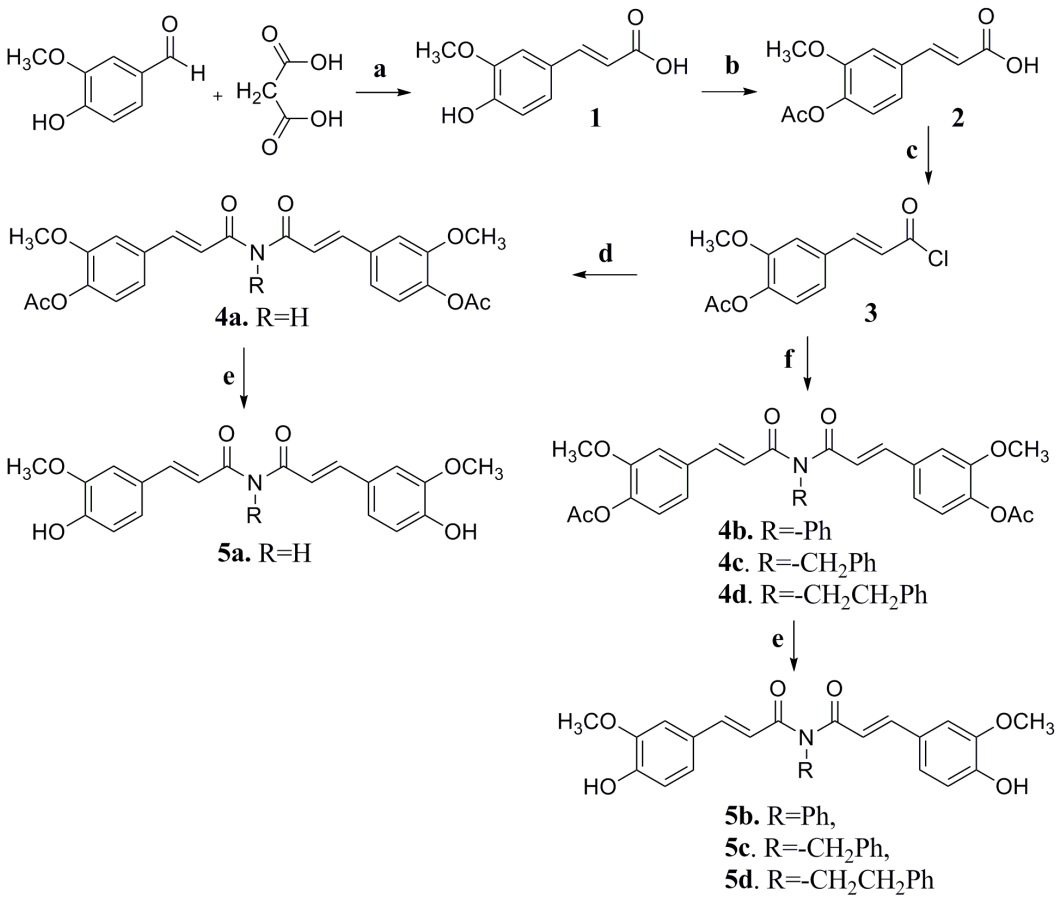

Scheme 1. Reagents and conditions: (**a**) Pyridine, toluene, aniline, reflux, 4h; (**b**) Ac_2O, DMAP, 0-10°C; (**c**) Dry DCM, DMF, $(COCl)_2$, 0°C, 2h; (**d**) HMDS, TEA overnight reflux; (**e**) 4% NH_2NH_2in acetonitrile, 15-30 min, rt; (**f**). C_6H_5-NH_2/ C_6H_5-CH_2NH_2 / C_6H_5-$CH_2CH_2NH_2$, TEA, overnight reflux

with HMDS in the presence of a base triethylamine in dicloromethane.[31] Synthesis of **4b-4d** wereachieved by following similar route but by replacing HMDS with appropriate amines. The final compounds **5a-5d** were obtained by the deacetylation of **4a-4d** with 4% hydrazine hydrate in acetonitrile.[37] Formation of ferulic acid (**1)** and isoferulic acid (**2)** were confirmed by their melting points 170-173 °C (Lit. 168-172 °C)[34] and 200 °C (Lit. 199-200 °C),[35] respectively. The final compounds **5a-5d** were characterized by their ^{1}HNMR, ^{13}CNMR and MS analysis (in Supplementary material). All the compounds displayed two singlet for two Ar–O$C\underline{H}_3$ group that appeared between δ: 3.68-3.84 ppm. Compound **5a** displayed singlet for –N$\underline{H}$- protons at δ: 12.22 ppm. The olefinic protons appeared between δ: 6.35-7.84 ppm as doublets. ESI-MS spectra of all the final compounds displayed the characteristic molecular ion peak (M^+).

2.2. Biochemistry

The compounds **5a-5d** were screened for h-MAO inhibitory activity by method reported earlier.[38-42] All are found to be selective, competitive and reversible inhibitors of hMAO-A isoform (**Table 1**). A steady increase in potency has been realized from **5a** to **5d**. While comparing the activity of **5a** with **5b-5d,** we observe 3.34-, 10.63- and 19.5-fold increase in potency, respectively. Replacement of H of **5a** with phenyl ring (**5b)** rendered 3.34-fold increase in potency, while replacement of (i) phenyl of **5b** with benzyl (**5c**) and (ii) benzyl of **5c** with phenylethyl (**5d**) rendered 3.18 and 1.83-fold increase in potency, respectively. Thus subsequent replacements provided slightly decreasing fold in potency. Compound **5d** displayed highest potency against hMAO-A isoform with Ki value of 0.06±0.002 µM and selectivity index of 507.33.

Surprisingly on the other hand the activity against hMAO-B isoforms also displayed the similar trend. A steady increase in potency is observed from **5a** to **5d**. Amongst these four compounds, **5d** has shown highest potency against hMAO-B isoform with Ki value of 30.44±10.08 µM. Compared with **5a**, there is 4.25-, 5.27 and 15.29-fold increase in potency for **5b, 5c** and **5d**, respectively. Subsequent replacements rendered 4.25-, 1.24- and 2.89-fold increase in potency. This may be due to different orientation of compounds in hMAO-B active site.

The compounds **5a** and **5d** were further analyzed for their permeability and metabolic stability by *in vitro* Caco-2 permeability assay[43-45] and human liver microsomal stability assay[46, 47] methods. The results are presented in **Table2** and **3**. Due to poor detection sensitivity values for **5d** could not be determined. With respect to **5a**, there is no improvement in permeability but a slight improvement in metabolic stability is observed as compared with curcumin. This suggests that modifications rendered the molecule to have an improved metabolic stability. As values for **5d** could not be determined, a comment on permeability enhancement is not feasible here, except that

Table 1. Experimental K_i values corresponding to the inhibition of hMAO isoforms by compounds **5a-5d**

Code	R	K_i value (μM)**		MAO-A SI*	Inhibition type	Reversibility	MAO selectivity
		MAO-A	MAO-B				
5a	H	1.17±0.10	465.50±20.55	398	Competitive	Reversible	MAO-A
5b	-Ph	0.35±0.02	109.55±5.26	313	Competitive	Reversible	MAO-A
5c	$-CH_2Ph$	0.11±0.01	88.26±5.90	802.36	Competitive	Reversible	MAO-A
5d	$-CH_2CH_2Ph$	0.06±0.002	30.44±10.08	507.33	Competitive	Reversible	MAO-A
Curc	-	0.71±0.04	21.50±1.19	30.28	Competitive	Reversible	MAO-A
Sel	-	8.96±1.33	0.10±0.09	0.01	Competitive	Irreversible	MAO-B
Moc	-	0.013±0.009	1.35±0.20	103.84	Competitive	Reversible	MAO-A

*Selectivity index calculated with Experimental Ki values: It was calculated as Ki (MAO-A)/Ki (MAO-B) for MAO-B and Ki (MAO-B)/Ki (MAO-A) for MAO-A. Selectivity towards MAO isoforms increases as the corresponding SI decreases. **Each value represents the mean±SEM of three independent experiments. Curc-Curcumin; Sel-Selegiline; Moc-Moclobemide.

Table 2. *In vitro* Caco-2 permeability data

Code	Avg P_{app} x 10^{-6} cm/sec (n = 2)			Permeability rank
	A → B	B → A	ER = B → A / A → B	
5a	0	8.8	NA	Low
5d	ND*	ND*	ND*	ND*
Curc	0	0	NA	Low

NA: Not Applicable; ND*: Not determined due to poor detection sensitivity; A → B: Apical to Basal; B → A: Basal to Apical; Permeability ranking based on following criteria (In-house): Low: P_{app} A-B < 5, High: P_{app} A-B ≥ 5. Curc-Curcumin

Table 3. *In vitro* human liver microsomal stability data

Code	T-half (min)	CLint, app (μL/min/m)
5a	11.4	121.8
5d	ND*	ND*
Curc	7.7	180.33

ND*: Not determined due to poor detection sensitivity; LM conc: 0.5mg/mL; T-half range: 3-120 min; CLint, app range: 11.6-462.0 μL/min/mg; Curc-Curcumin

bioisosteric replacement of $-CH_2-$ with -NH- did not improved permeability.

3. Experimental Section:

Materials and Methods: All the chemicals and solvents for synthesis were purchased from Aldrich. Unless otherwise mentioned the solvents were used without purification. Reactions were monitored by TLC on pre-coated silica gel plates (Kieselgel 60 F 254, Merck) and the spots were detected under UV light (254 nm). Purification was performed by column chromatography using silica gel (particle size 100-200 mesh, CDH). Melting points were determined using Optimelt (Stanford Research Systems, Sunnyvale, CA 94089) by capillary method and are uncorrected. ^{1}HNMR & ^{13}CNMR spectra were recorded on Varian 400 & Varian 100 MHz instruments, respectively in DMSO-d_6. Chemical shifts were reported in parts per million (ppm) downfield with respect to tetramethylsilane (TMS, δ = 0.0) as internal standard. Coupling constants (*J*) were given in hertz (Hz). Mass spectra were recorded on ESI-MS (Electro spray Ionization-Mass spectra). Enzymes hMAO-A and hMAO-B (both recombinant, expressed in baculovirus-infected BTI insect cells), and other chemicals were purchased from Sigma-Aldrich (Munich, Germany). The Amplex®-Red MAO assay kit was purchased from Cell Technology Inc., Mountain View, CA, USA.

3.1. Synthesis:

Procedure for the synthetic of ferulic acid (1)

Vanillin (0.1 M) and malonic acid (0.106 M) were added to a mixture of toluene (15 mL), pyridine (0.153 M) and aniline (0.011M). The solution was stirred at refluxing temperature for 2 h. When the mixture was cooled to room temperature, yellow precipitate was obtained. It was then filtered and washed with 50 mL of 3M HCl followed by 50 mL of water twice. The crude product was further recrystallized from ethanol.[35]

Procedure for the synthesis of acetylated ferulic acid (2)

In a 250 mL three-necked round bottom flask with a cooling apparatus, ferulic acid (**1,** 0.084 M) and 4-*N, N*-dimethyl amino pyridine (0.084 M) in 50 mL of acetic anhydride was taken. After stirring the reaction mixture at room temperature for 3 h, the mixture was diluted with 100 mL of water. Stirring was continued for overnight and filtered. The obtained solid (**2**) was washed with water.[34]

Procedure for chlorination of O-acetyl ferulic acid (3)

The solution of acetylated ferulic acid (**2,** 0.01 M) in dry dichloro methane (10 mL), oxalyl chloride (0.02 M) and DMF (25 μL) were added dropwise. The reaction mixture was then stirred under argon atmosphere for 15 min at room temperature. The solvent was removed under vacuum to provide acetylated feruloyl chloride (**3**) as a pale yellow solid.[36]

Synthesis of (E)-3-(4-hydroxy-3-methoxyphenyl)-N-((E)-3-(4-hydroxy-3-methoxyphenyl) acryloyl) acrylamide (4a)

To a refluxing solution of acetylated feruloyl chloride (**3,** 0.1 M) in 10 mL anhydrous tetrahydro furan, HMDS (0.05 M) was added dropwise. Finally triethyl amine (0.07 M) was added to the above refluxing mixture and allowed to reflux overnight. The solvent was removed under vacuum. The obtained solid was then extracted with 3x25 mL of dichloro methane. The organic layer was further washed with 3x50 ml of water followed by brine, and dried over anhydrous sodium sulfate to obtain crude product and was further purified by column chromatography.[31]

General procedure for the synthesis of 4b-4d

To a refluxing solution of acetylated feruloyl chloride (**3**, 0.1 M) in10 mL anhydrous tetrahydro furan, appropriate amine (0.05 M) was added dropwise. Finally triethyl amine (0.07 M) was added dropwise and allowed to reflux overnight. The solvent was removed under vacuum. The obtained solids were extracted with 3x25 mL of dichloro methane. The organic layer was

further washed with 3x50 mL of water followed by brine, and dried over anhydrous sodium sulfate to obtain crude products (**4b-4d**). Those were further purified by column chromatography.

General procedure for the synthesis of 5a-5d

To a solution of **4a-4d** in 10 mL acetonitrile was added hydrazine monohydrate (4%) in acetonitrile. The mixture was then stirred for about 2 h at room temperature. The organic portion was extracted with ethyl acetate, washed with water, brine and dried over anhydrous sodium sulphate. After the solvent was evaporated, the product was purified by column chromatography using $CHCl_3$/MeOH to afford compounds **5a-5d.**[37]

3-(4-hydroxy-3-methoxyphenyl)-N-((E)-3-(4-hydroxy-3-methoxyphenyl)-acryloyl) acrylamide (5a)

Yield: 20%; m.p: 151 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ (ppm) 12.12 (s, 1H, -NH-), 9.55 (s, 2H, -OH), 7.49 (d, 2H, J=12 Hz), 7.28 (s, 2H, Ar-H), 7.08 (d, 2H, J=8 Hz, Ar-H), 6.79 (d, 2H, J=8 Hz), 6.36 (d, 2H, J=20 Hz), 3.81 (s, 6H, -OCH_3); ESI-MS: m/z=369.6.

3-(4-hydroxy-3-methoxyphenyl)-N-((E)-3-(4-hydroxy-3-methoxyphenyl)acryloyl)-N-phenyl acrylamide (5b)

Yield: 40%; m.p: 120 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ (ppm) 3.83 (s, 3H, -OCH_3), 3.84 (s, 3H, -OCH_3), 6.35 (d, 1H, J=16 Hz), 6.85-6.82 (m, 1H), 7.06 (t, 2H, J=8 Hz, 4 Hz), 7.25-7.15 (m, 2H), 7.36-7.30 (m, 3H), 7.39 (s, 1H-Ar-H), 7.5 (d, 1H, J=16 Hz), 7.6 (d, 1H, *J*=16 Hz), 7.71 (t, 3h, J=4 Hz, 8 Hz), 9.55 (s, 1H, -OH), 10.23 (s, 1H, -OH); ESI-MS (m/z): 445.9.

N-benzyl-3-(4-hydroxy-3-methoxyphenyl)-N-((E)-3-(4-hydroxy-3-methoxyphenyl)-acryloyl) acrylamide (5c)

Yield: 45%; m.p: 122 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ (ppm) 3.68 (s, 3H, -OCH_3) 3.81 (s, 3H, -OCH_3), 4.405 (d, 2H, J=4 Hz, -CH_2-), 5.16 (s, -OH), 6.7 (d, 1H, J=16 Hz), 7.12 (d, 1H, J=8 Hz), 7.17 (d, 1H, J=8.4 Hz), 7.36 (m, 9H), 7.41 (s, 1H, Ar-H), 7.47 (d, 1H, J=16 Hz), 7.64 (d, 1H, J=16 Hz), 8.62 (t, 1H); ^{13}CNMR (400 MHz, DMSO-d_6): δ (ppm) 168.918, 165.336, 151.495, 128.790, 134.360, 138.822, 139.808, 140.582,127.826, 127.310, 123.721, 122.795, 120.511, 111.989, 56.198, 42.781, 20.850; ESI-MS (m/z): 459.8.

3-(4-hydroxy-3-methoxyphenyl)-N-((E)-3-(4-hydroxy-3-methoxyphenyl)-acryloyl)-N-phenethyl acrylamide (5d)

Yield: 35%; m.p: 105 °C; ^{1}HNMR (400 MHz, DMSO-d_6): δ (ppm) 2.78 (t, 2H, J=8 Hz, 4 Hz, -CH_2-), 3.40-3.45 (m, 2H, J=8 Hz, 4 Hz, -CH_2-) 3.81 (s, 3H, -OCH_3), 3.82 (s, 3H, -OCH_3), 6.64 (m, 1H), 6.98 (d, 1H, J=16 Hz), 7.25-7.21 (m, 3H), 7.1 (d, 1H, J=8 Hz), 7.15 (d, 1H, J=8 Hz), 7.30 (3H, J=8 Hz), 7.41 (d, 1H, J=16 Hz), 7.49 (s, 1H, Ar-H), 7.58 (d, 1H, J=16 Hz), 7.84 (d, 1H, J=16 Hz), 8.19 (t, 1H), 12.42 (s, 1H, -OH); ESI-MS (m/z): 473.2.

3.2. Biochemistry:

3.2.1. Determination of hMAO-A and -B activities:

Human MAO-A and MAO-B activities were determined using *p*-tyramine. The inhibitory effects of the synthesized compounds on the MAO activities were determined by a fluorimetric method described previously.[38-42] The production of H_2O_2 catalyzed by MAO was detected using the Amplex®-Red reagent, a non-fluorescent, highly sensitive, and stable probe that reacts with H_2O_2 in the presence of horseradish peroxidase to produce the fluorescent product resorufin. The reaction was started by adding (final concentrations) 200 μM Amplex®-Red reagent, 1 U/mL horseradish peroxidase, and *p*-tyramine (concentration range, 0.1-1 mM). Control experiments were carried out by replacing the synthesized compound and reference inhibitors (selegiline and moclobemide) in the presence of *p*-tyramine. The possible capacity of the novel compounds to modify the fluorescence generated in the reaction mixture due to non-enzymatic inhibition was determined by adding these compounds to solutions containing only the Amplex®-Red reagent in sodium phosphate buffer.

3.2.2. Kinetic experiments:

The synthesized compounds were dissolved in DMSO with a maximum concentration of 1% (w/v) and used in the concentration range of 1-100 μM. The specificity index was expressed as $SI=K_i$ (MAO-A)/K_i(MAO-B). Protein was determined according to Bradford[48] using bovine serum albumin as the standard.

3.2.3. Reversibility experiments:

Reversibility was assessed using a centrifugation-ultra filtration method previously reported.[49] Recombinant enzymes were incubated with the compounds or standard inhibitors in 0.05 M sodium phosphate buffer, pH 7.4, for 1 h at 37 °C. An aliquot was stored at 4 °C for MAO activity measurement. Another sample was placed in an Ultrafree-0.5 centrifuge tube (Nalgene®, NY, USA) with a 30-kDa Biomax membrane (Millipore Corp., Bedford, MA, USA) and centrifuged at 9000 x *g* for 20 min at 4 °C. The enzyme retained in the 30-kDa membrane was resuspended in the buffer at 4 °C and centrifuged, and this process was repeated once more. The enzyme retained in the membrane was resuspended in buffer and used for MAO determination. Control experiments were performed by replacing the test drugs with appropriate vehicle dilution. The corresponding values of percent MAO inhibition were separately calculated for the samples with and without repeated washing.

3.3. *In vitro* permeability and metabolic stability studies

In vitro permeability studies using Caco-2 cell lines and metabolic stability studies using human liver microsomal enzymes were carried out as per the procedure reported by Mondal et al.,[50, 51]

Abbreviations:

MAO: Monoamine oxidase; hMAO: human MAO; rMAO: rat MAO; SAR: Structure-activity relationship; HMDS: Hexamethyl disilazane.

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References

1. Maheshwari, R. K.; Singh, A. K.; Gaddipati, J.; Srimal, R. C., Multiple biological activities of curcumin: a short review. *Life Sci* 2006, 78, 2081-2087.
2. Goel, A; Kunnumakkara, A. B.; Aggarwal, B. B., Curcumin

as ''Curecumin'': From kitchen to clinic. *Biochem Pharmacol* 2008, 75, 787-809.
3. Aggarwal, B. B.; Kuzhuveli, B. H., Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. *Int J Biochem Cell Biol* 2009, 41, 40-59.
4. Ali, N.; Ashkani-Esfahani, S., A Review of Therapeutic Effects of Curcumin. *Curr Pharm Des* 2013, 19, 2032-2046.
5. Dulbecco, P.; Savarino, V., Therapeutic potential of curcumin in digestive diseases. *World J Gastroenterol* 2013, 19, 9256-9270.
6. Lestari, M. L. A. D.; Gunawan, I., Curcumin. Profiles of Drug Substances, Excipients, and Related Methodology 2014, 39, 113-204.
7. Xu, Y.; Ku, B.S.; Yao, H.Y.; Lin, Y.H.; Ma, X.; Zhang, Y.H.; Li, X.J., The effects of curcumin on depressive-like behaviors in mice. *Eur J Pharmacol* 2005, 518, 40–46.
8. Xu, Y.; Ku, B.S.; Yao, H.Y.; Lin, Y.H.; Ma, X.; Zhang, Y.H.; Li, X.J., Antidepressant effects of curcumin in the forced swim test and olfactory bulbectomy models of depression in rats. *Pharmacol Biochem Behav* 2005, 82, 200-206.
9. Wang, R.; Xu, Y.; Wu, H.L.; Li, Y.B.; Li, Y.H.; Guo, J.B.; Li, X. J., The antidepressant effects of curcumin in the forced swimming test involve 5-HT1 and 5-HT2 receptors, *Eur J Pharmacol* 578 (2008) 43–50.
10. Kulkarni, S.K.; Bhutani, M.K.; Bishnoi, M., Antidepressant activity of curcumin: Involvement of serotonin and dopamine system. *Psychopharmacol* 2008, 201, 435–442.
11. Xu, Y.; Ku, B.; Lu, T.; Yao, H.; Jiang, W.; Ma, X.; Li, X., Curcumin reverses the effects of chronic stress on behavior, the HPA axis, BDNF expression and phosphorylation of CREB. *Brain Res* 2006, 1122, 56-64.
12. Xu, Y.; Ku, B.; Cui, L.; Li, X.; Barish, P. A.; Foster, T. C.; Ogle, W. O., Curcumin reverses impaired hippocampal neurogenesis and increases serotonin receptor 1A mRNA and brain-derived neurotropic factor expression in chronically stressed rats. *Brain Res* 2007, 1162, 9-18.
13. Kulkarni, S. K.; Dhir, A.; Akula, K. K., Potentials of Curcumin as an Antidepressant. *Sci World J* 2009, 9, 1233-1241.
14. Gradowski, R. W.; Santiago, R. M.; Zaminelli, T.; Bassani, T. B.; Barbiero, J. K.; Boschen, S. L.; Andreatini, R.; Vital, M. A. B. F., Antidepressant-like effect of curcumin in 6-hydroxydopamine model of Parkinson's disease. *Curr Trends Neurol* 2013, 7, 69-80.
15. Jiang, H.; Wang, Z.; Wang, Y.; Xie, K.; Zhang, Q.; Luan, Q.; Chen, W.; Liu, D., Antidepressant-like effects of curcumin in chronic mild stress of rats: Involvement of its anti-inflammatory action. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2013, 47, 33-39.
16. Witkin, J. M.; Leucke, S.; Thompson, L. K.; Lynch, R. A.; Ding, C.; Heinz, B.; Catlow, J. T.; Gleason, S. D.; Li, X., Further evaluation of the neuropharmacological determinants of the antidepressant-like effects of curcumin. *CNS Neurol Disord Drug Targets* 2013, 12, 498-505.
17. Zhang, L.; Xu, T.; Wang, S.; Yu, L.; Liu, D.; Zhan, R.; Yu, S. Y., NMDA GluN2B receptors involved in the antidepressant effects of curcumin in the forced swim test. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2013, 40, 12-17.
18. Zhao, X.; Wang, C.; Zhang, J.-F.; Liu, L.; Liu, A.-M.; Ma, Q.; Zhou, W.-H.; Xu, Y., Chronic curcumin treatment normalizes depression-like behaviors in mice with mononeuropathy: involvement of supraspinal serotonergic system and GABAA receptor. *Psychopharmacol* 2014, 231, 2171-2187.
19. Anand, P.; Kunnumakkara, A. B.; Newman, R. A.; Aggarwal, B. B., Bioavailability of Curcumin: Problems and Promises. *Mol Pharmaceutics* 2007, 4, 807-818.
20. Berginc, K.; Trontelj, J.; Basnet, N. S.; Kristl, A., Physiological barriers to the oral delivery of curcumin. Pharmazie 2012, 67, 518-524.
21. Begum, A. N.; Jones, M. R.; Lim, G. P.; Morihara, T.; Kim, P.; Heath, D. D.; Rock, C. L.; Pruitt, M. A.; Yang, F.; Hudspeth, B.; Hu, S.; Faull, K. F.; Teter, B.; Cole, G. M.; Frautschy, S. A., Curcumin structure-function, bioavailability, and efficacy in models of neuroinflammation and Alzheimer's disease. *J Pharmacol Exp Ther* 2008, 326, 196-208.
22. Wang, Q.; Wang, K., Metabolism of curcumin. *Zhongguo Yaolixue Tongbao* 2003, 19, 1097-1101.
23. Metzler, M.; Pfeiffer, E.; Schulz, S. I.; Dempe, J. S., Curcumin uptake and metabolism. *Biofactors* 2013, 39, 14-20.
24. Yallapu, M. M.; Jaggi, M.; Chauhan, S. C., Curcumin nanoformulations: a future nanomedicine for cancer. *Drug Disc Today* 2012, 17, 71-80.
25. Dagar, P.; Dahiya, P.; Bhambi, M., Recent advances in curcumin nanoformulations. *Nano Sci Nano Technol Indian J* 2014, 8, 458-474.
26. Prasad, S.; Tyagi, A. K.; Aggarwal, B. B., Recent Developments in Delivery, Bioavailability, Absorption and Metabolism of Curcumin : the Golden Pigment from Golden Spice. *Cancer Res and Treat* 2014, 46, 2-18.
27. Singh, P. K.; Wani, K.; Kaul-Ghanekar, R.; Prabhune, A.; Ogale, S., From micron to nano-curcumin by sophorolipid co-processing: highly enhanced bioavailability, fluorescence, and anti-cancer efficacy. *RSC Adv* 2014, 4, 60334-60341.
28. Tian, F.; Hu, J., Pharmacokinetic features of curcumin and research progress in the drug form modification. *Yaoxue Fuwu Yu Yanjiu* 2014, 14, 458-461.
29. Vyas, A.; Dandawate, P.; Padhye, S.; Ahmad, A.; Sarkar, F., Perspectives on New Synthetic Curcumin Analogs and their Potential Anticancer Properties. *Curr Pharm Des* 2013, 19, 2047-2069.
30. Chakraborti, S.; Dhar, G.; Dwivedi, V.; Das, A.; Poddar, A.; Chakraborti, G.; Basu, G.; Chakrabarti, P.; Surolia, A.; Bhattacharyya, B., Stable and Potent Analogues Derived from the Modification of the Dicarbonyl Moiety of Curcumin. *Biochemistry* 2013, 52, 7449-7460.
31. Lin, L.; Shi, Q.; Nyarko, A. K.; Bastow, K. F.; Wu, C. C.; Su, C. Y.; Shih, C. C. Y.; Lee, K. H., Antitumor Agents. 250.Design and Synthesis of New Curcumin Analogues as Potential Anti-Prostate Cancer Agents. *J Med Chem* 2006, 49, 3963-3972.
32. Banerjee, S.; Chakravarty, A. R., Metal Complexes of Curcumin for Cellular Imaging, Targeting, and Photoinduced Anticancer Activity. *Acc Chem Res* 2015, 48, 2075-2083.
33. Chen, Q. H. Preparation of curcumin analogs for the treatment of prostate cancer. US20150017720A1, 2015.
34. Xia, C. N.; Li, H. B.; Liu, F.; Hu, W. X., Synthesis of trans-caffeate analogues and their bioactivities against HIV-1 integrase and cancer cell lines. *Bioorg Med Chem Lett* 2008, 18, 6553-6557.
35. Galey, J. B.; Terranova, E. Mono- and di-esters of cinnamic acid or its derivatives with vitamin C, process for their preparation, and their use as antioxidants in cosmetic, pharmaceutical, or alimentary compositions. EP664290A1, 1995.
36. Ergun, B. C.; Coban, T.; Onurdag, F. K.; Banoglu, E., Synthesis, antioxidant and antimicrobial evaluation of simple aromatic esters of ferulic acid. *Arch Pharmacal Res* 2011, 34, 1251-1261.
37. Nomura, E.; Kashiwada, A.; Hosoda, A.; Nakamura, K.; Morishita, H.; Tsuno, T.; Taniguchi, H., Synthesis of amide compounds of ferulic acid, and their stimulatory effects on insulin secretion *in vitro*. *Bioorg Med Chem* 2003, 11, 3807-3813.
38. Yanez, M.; Fraiz, N.; Cano, E.; Orallo, F., Inhibitory effects of cis-and trans-resveratrol on noradrenaline and 5-hydroxytryptamine uptake and on monoamine oxidase activity. *Biochem Biophys Res Commun* 2006, 344, 688-695.
39. Chimenti, F.; Maccioni, E.; Secci, D.; Bolasco, A.; Chimenti, P.; Granese, A.; Carradori, S.; Alcaro, S.; Ortuso, F.; Yá-ez, M., Synthesis, stereochemical identification, and selective inhibitory activity against human monoamine oxidase-B of 2-methylcyclohexylidene-(4-arylthiazol-2-yl) hydrazones. *J Med Chem* 2008, 51, 4874-4880.
40. Badavath, V. N.; Yabanoglu, S. C.; Bhakat, S.; Timiri, A. K. T.; Sinha,B. N.; Ucar, G.; Soliman, M. E. S.; Jayaprakash, V.,

Monoamine oxidase inhibitory activity of 2-aryl-4H-chromen-4-ones. *Bioorg Chem* 2015, 58, 72–80.

41. Badavath, V. N.; Yabanoglu, S. C.; Bhakat, S.; Jadav, S. S.; Jagarat, M.; Sinha, B. N. ; Ucar, G.; Jayaprakash, V., Monoamine oxidase inhibitory activity of 3, 5-biaryl-4,5-dihydro-1Hpyrazole-1-carboxylate derivatives. *Eur J Med Chem* 2013, 69, 762-767
42. Badavath, V. N.; Alok, K.; Jadav, S. S.; Mishra, N.; Dev, A.; Sinha, B. N.; Jayaprakash, V., Pyrazoline carboxylates as selective MAO-B inhibitors: Synthesis and Biological screening. *J Pharm Chem* 2015, 2, 1-5.
43. Hitzel, L.; Watt, A.; Locker, K., An Increased Throughput Method for the Determination of Partition Coefficients. *Pharm Res* 2000, 17, 1389-1395.
44. Kerns, E. H.; Di, L.; Petusky, S.; Farris, M.; Ley, R.; Jupp, P., Combined application of parallel artificial membrane permeability assay and Caco-2 permeability assays in drug discovery. *J Pharm Sci* 2004, 93, 1440-1453.
45. Balimane, P.; Han, Y.-H.; Chong, S., Current industrial practices of assessing permeability and P-glycoprotein interaction. *AAPS J* 2006, 8, E1-E13.
46. Di, L.; Kerns, E. H.; Hong, Y.; Kleintop, T. A.; Mc Connell, O. J.; Huryn, D. M., Optimization of a Higher Throughput Microsomal Stability Screening Assay for Profiling Drug Discovery Candidates. *J Biomol Screen* 2003, 8, 453-462.
47. Jenkins, K. M.; Angeles, R.; Quintos, M. T.; Xu, R.; Kassel, D. B.; Rourick, R. A., Automated high throughput ADME assays for metabolic stability and cytochrome P450 inhibition profiling of combinatorial libraries. *J Pharm Biomed Anal* 2004, 34, 989-1004.
48. Bradford, M. M., A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976, 72, 248-254.
49. Chimenti, F.; Carradori, S.; Secci, D.; Bolasco, A.; Bizzarri, B.; Chimenti, P.; Granese, A.; Yá-ez, M.; Orallo, F., Synthesis and inhibitory activity against human monoamine oxidase of N1-thiocarbamoyl-3, 5-di (hetero) aryl-4, 5-dihydro-(1H)-pyrazole derivatives. *Eur J Med Chem* 2010, 45, 800-804.
50. Mondal, S. K.; Upal, K. Mazumder.; Mondal, N. B.; Banerjee, S Mazumder., Optimization of rat liver microsomal stability assay using HPLC. *J Biol Sci* 2008, 8, 1110-1114.
51. Mondal, S. K.; Mondal, N. B.; Banerjee, S Mazumder.; Upal, K. Mazumder., Determination of drug-like properties of a novel antileishmanial compound: In vitro absorption, distribution, metabolism, and excretion studies. *Indian J Pharmacol* 2009, 41,176-81.