New Bioactive Esters and Phosphonates Semisynthesized From (±)-Vasicinone: An Alkaloid Isolated From Peganum harmala

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Abstract
A series of $N$-tosyl $\alpha$-amino acids 2a-e, prepared using a tosyl chloride protecting group, was condensed with (±)-vascinone 1, isolated from the seeds of the plant Peganum harmala, to generate the corresponding esters 3a-e and 3b'–e'. (±)-Vasicinone 1 was also reacted with chloroacetic acid chloride to afford a new chlorinated ester 4 which was refluxed with trialkyl phosphites to give 2 new phosphonates 5a,b. All synthesized compounds were characterized with the help of spectroscopic means, including NMR ($^1$H, $^{13}$C, and $^{31}$P) and ES-HRMS, and then screened for their in vitro anti-acetylcholinesterase (AChE), anti-5-lipoxygenase (5-LOX), and cytotoxic activities (MCF-7, OVCAR-3, and HCT-116 cell lines). Most synthesized derivatives exhibited a cytotoxic activity against 3 cell lines used. The phosphonate derivative 5b was found to be the most active one ($IC_{50} = 63.7 \pm 1.4 \mu M$) against AChE enzyme. Only 2 diastereoisomers 3e and 3e' exhibited activity against 5-LOX enzyme with IC\textsubscript{50} values of 63.1 ± 4.2 and 79.2 ± 8.3 µM, respectively.

Keywords
(±)-vascinone, structural analogs, $\alpha$-amino esters, phosphonate esters, bioactivity

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In view of the above observations and as a continuation of our previous work on the synthesis of new structural analogs of alkaloids isolated from P. harmala seeds, we report here the synthesis of new series of $\alpha$-amino acid ester and phosphonate derivatives of (±)-vascinone. Furthermore, the newly synthesized compounds were tested in vitro for their acetylcholinesterase (AChE) and 5-lipoxygenase (5-LOX) inhibitor potentials and cytotoxic activity against MCF-7 (Human breast carcinoma), OVCAR-3 (human ovarian carcinoma), and HCT-116 (Human colon carcinoma).

(±)-Vasicinone 1 was isolated from the seeds of P. harmala adopting the method described in our previous work, and

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identified by spectroscopic means including NMR analysis (1H and 13C NMR) and HRMS, as well as by comparison with literature data.10

As illustrated in Scheme 1 and in order to prepare new ester derivatives consisting of both (±)-vasicinone and α-amino acids, we started by the protection of the amino group in the α-amino acids using tosyl chloride to generate N-tosyl α-amino acids 2a-e. The structures of these compounds were confirmed according to their spectral data.

Then, (±)-vasicinone 1 was reacted with the series of N-tosyl α-amino acids 2a-e in reflux of anhydrous toluene in the presence of p-toluenesulfonic acid to afford a mixture of 2 diastereoisomers that were successfully separated by column chromatography on silica gel to generate the corresponding esters 3a-e and 3b′-e′ (Scheme 1) and differentiated by their retention times by means of RP-HPLC (Reversed Phase-High Pressure Liquid Chromatography) analysis using acetonitrile and H2O as mobile phase (Table 1). Due to the lack of amounts, it was not possible to measure their optical rotations.

On the other hand, encouraged by our results and in conjunction with the pharmaceutical importance known for fused heterocyclic incorporating a phosphonate moiety, we decided

### Table 1. Yield (%) and HPLC Retention Time t_r (min) of Derivatives 3a-e and 3b′-e′

| Entry | Compounds | R       | Yield (%) | Diast. I t_r (min) | Diast. II t_r (min) | (d.e) |
|-------|-----------|---------|-----------|--------------------|---------------------|-------|
| 1     | 3a        | -H      | 70 (a)    | 12.89              | -                   |       |
| 2     | 3b,3b′    | -CH₃    | 37 (b), 12.37 | 23 (b′), 12.78    | 14                  |
| 3     | 3c,3c′    | CH₂-iPr | 23 (c), 13.14 | 22 (c′), 13.35     | 2                   |
| 4     | 3d,3d′    | -Ph     | 30 (d), 13.13 | 32 (d′), 13.21     | 2                   |
| 5     | 3e,3e′    | CH₂-Ph  | 70 (e), 12.71 | 30 (e′), 13.02     | 40                  |

^The yield was determined after purification.

^The d.e (diastereoisomeric excess) was measured from the 1H NMR spectra of the crude reaction.

Scheme 1. Synthetic pathway to compounds 2a-e, 3a-e, and 3b′-e′.
to continue to explore the secondary alcohol function of (+)-vasicinone 1 wishing to synthesize new structural derivatives which can display interesting biological activities. Our approach to the target fused (+)-vasicinone-phosphonate derivatives firstly started by the preparation of the chlorinated derivative 4 through the esterification of (+)-vasicinone 1 with chloroacetic acid chloride in the presence of triethylamine (Scheme 2) to give the intermediate 4 (Table 2). The structure of this compound was confirmed on the basis of its spectral data (ES-HRMS; $^1$H and $^{13}$C NMR). Then, as shown in Scheme 2, the chlorinated ester 4 was refluxed with an excess of an appropriate trialkyl phosphite during 12 hours to generate the corresponding (+)-vasicinone-phosphonate derivatives 5a,b (Table 2). Their structures were established on the basis of their spectral data (ES-HRMS; $^1$H, $^{13}$C, and $^{31}$P NMR). The $^{13}$C NMR spectrum of compound 5a, as an example, shows the appearance of a doublet at $\delta_C$ 54.5 ($J_{C,P} = 98.7$ Hz) in agreement with the presence of 2 methoxyl groups attached to the phosphorus atom. Moreover, the $^{31}$P NMR spectrum of compound 5a showed the presence of a singlet at $\delta_P$ 21.9 relative to the phosphorus atom introduced by the trimethyl phosphate.

Finally, the ES-HRMS mass spectra of all the synthesized compounds (3a-e, 3b'-e', 4, and 5a,b) were in agreement with their molecular formula.

In order to survey the possible biological activities of (+)-vasicinone 1 and its newly prepared derivatives 3a-e, 3b'-e', and 5a,b, they were screened for their cytotoxic activity against 3 cancer cell lines: OVCAR-3, ovarian cancer cell line; MCF-7, a breast cancer cell line; and HCT-116, a human colon cancer cell line, as well as for their anti-acetylcholinesterase and anti-5-LOX potentials.

The cytotoxic assay (Table 3) showed that (+)-vasicinone 1 did not display any proliferation inhibition of MCF-7, OVCAR-3, and HCT-116 cell lines (IC$_{50} > 100$ µM). However, the ester derivatives 3a-e and 3b'-e' have shown variable inhibitory potentials toward these cell lines. It was also noted that the esters 3b, 3b', 3c, and 3c' were found to be the most cytotoxic derivatives (Table 3). The results showed that these 4 compounds exhibited comparable cytotoxic activity against HCT-116 cell line (IC$_{50} = 36 \pm 2.32 \pm 4$ µM) and toward OVCAR-3 cell line (IC$_{50} = 59 \pm 8.51 \pm 9$ µM). On the other hand, the results showed that 3b' (IC$_{50} = 38 \pm 2$ µM) and 3c (IC$_{50} = 53 \pm 3$ µM) were found to be more active than their diastereoisomers 3b (IC$_{50} = 51 \pm 4$ µM) and 3c' (IC$_{50} = 56 \pm 4$ µM), respectively, against MCF-7 cell line. This finding clearly shows the intervention of the stereochemistry of these compounds in the inhibition of this cell line.

| Entry | Product | R     | Yields (%) |
|-------|---------|-------|------------|
| 1     | 4       | -     | 40         |
| 2     | 5a      | -CH$_3$ | 50         |
| 3     | 5b      | -C$_2$H$_5$ | 90         |

*The yield was determined after purification.*
The relatively high activity of compounds 3a-a' and 3b-b' compared to that of their analogs 3c-c' and 3d-d' demonstrates that this biological effect depends on the nature of the R group fixed at C-2 position of the α-amino acid used. In fact, it has been found that the methyl group in 3b-b' favors the cytotoxic activity against the cell lines used more than the sec-butyl (3c-c'), followed by the phenyl (3d-d') and then the benzyl (3e-e') ones (Table 3).

The results given in Table 3 show that phosphonate derivatives 5a,b were not found to be active against OVCAR-3 cell line (IC$_{50}$ >100 µM), whereas they displayed moderate activity against MCF-7 and HCT-116 cell lines with IC$_{50}$ values ranging from 78 ± 8 to 57 ± 4 µM, except 5b which did not exhibit any activity toward HCT-116 cell line. These findings show the selectivity of these 2 phosphonate derivatives in relation to the cell lines used.

On the other hand, the derivative 5a (R= CH$_3$) was found to be active against HCT-116 with an IC$_{50}$ value of 57 ± 4 µM, while its analog 5b (R= C$_2$H$_5$) did not exhibit any activity against the same cell line (IC$_{50}$ >100 µM). Inversely, it has been noticed that 5b (IC$_{50}$ = 67 ± 9 µM) is relatively more active than its analog 5a (IC$_{50}$ = 78 ± 8 µM) against MCF-7 line. These findings showed the importance of the nature of the alkyloxy group attached to the phosphorus atom and its specific interaction with each cell line.

(±)-Vasicinone 1 and its derivatives, 3a-e, 3b'-e', and 5a,b, were further screened for their anti-AChE activity. As depicted in Table 4, (±)-vasicinone 1 and its ester derivatives (3a-e and 3b'-e') did not exhibit any considerable activity against AChE enzyme (IC$_{50}$ >100 µM).

For the phosphonate derivatives, only 5b was found to be active toward AChE enzyme with an IC$_{50}$ value of 63.7 ± 1.4 µM. This result compared to those of (±)-vasicinone 1 (IC$_{50}$ >100 µM) and compound 5a (IC$_{50}$ >100 µM) showed both the importance of the introduced phosphonate moiety and the nature of the alkyloxy attached to the phosphorus atom. However, it has been found that the presence of the ethoxy group (in 5b) is clearly in favor of this activity.

In summary, in this paper, we designed and synthesized a new series of α-amino esters 3a-e and 3b'-e', as well as 2 new phosphonates derivatives 5a,b from the natural alkaloid (±)-vasicinone 1 isolated from the seeds of P. harmala. The natural starting molecule and all the prepared analogs have been screened for their in vitro anti-AChE, anti-5-LOX, and cytotoxic activities against 3 cancer cell lines HCT-116, MCF-7, and OVCAR-3. The results showed that...
(±)-vasicinone 1 did not exhibit any cytotoxic, anti-AChE, and anti-5-LOX activities, whereas some of its ester derivatives 3a-e and 3b’-e’ displayed significant cytotoxic activity which depended on the nature of specific groups attached to the α-amino acids used (3b, b’ , c, c’e’, d, d’e’, and e ‘e’). Also, the configuration of asymmetric carbons has influenced this activity in some cases (3b, b’ and c, c’e’). On the other hand, it has been found that the addition of a phosphonate moiety to (±)-vasicinone (5a,b) led to get a cytotoxic activity against MCF-7 and HCT-116 cell lines for 5a,b and 5a, respectively, and an anti-AChE effect for 5a. These findings confirm in these cases the importance of this phosphonate system and the nature of its alkyloxy groups that could enter into any specific interactions with the cell lines used and AChE enzyme.

Experimental

Chemistry

Melting points were taken on a Buchi-510 capillary melting point apparatus. 1H (300 MHz) and 13C (75 MHz) NMR spectra were recorded on a Bruker AM-300 spectrometer, using CDCl3 and DMSO- d6 as solvent and nondeuterated residual solvent as internal standard. Chemicals shifts (δ) are given in parts per million (ppm) and coupling constants (J) in Hertz (Hz). High-resolution mass spectra were acquired with a Q TOF (Quadripole Time-of-Flight) 1er (Waters, ESI technique, positive mode). Preparative HPLC analysis was performed on an Ascentis Express C18, HPLC Column. 5 μm particle size, I x L D. 25 cm x 4.6 mm. Solvents (H2O, A and acetonitrile, B) were pumped by a Prostar two-way binary high-pressure gradient pump (Varian). UV detection was carried out at 240 and 280 nm by a Prostar 345 detector (Varian). The mobile phase was composed of 2 solvents: A, water (20%) and B, acetonitrile (80%). pH = 2.65 acetic acid. Degasser: ERC-3114; Ferryman sample: AS300, spectra were recorded on a Bruker AM-300 spectrometer, formed on an Ascentis Express C18, HPLC Column. 5 μm point apparatus. 1H (300 MHz) and 13C (75 MHz) NMR spectra were recorded on a Bruker AM-300 spectrometer, using CDCl3 and DMSO- d6 as solvent and nondeuterated residual solvent as internal standard. Chemicals shifts (δ) are given in parts per million (ppm) and coupling constants (J) in Hertz (Hz). High-resolution mass spectra were acquired with a Q TOF (Quadripole Time-of-Flight) 1er (Waters, ESI technique, positive mode). Preparative HPLC analysis was performed on an Ascentis Express C18, HPLC Column. 5 μm particle size, I x L D. 25 cm x 4.6 mm. Solvents (H2O, A and acetonitrile, B) were pumped by a Prostar two-way binary high-pressure gradient pump (Varian). UV detection was carried out at 240 and 280 nm by a Prostar 345 detector (Varian). The mobile phase was composed of 2 solvents: A, water (20%) and B, acetonitrile (80%). pH = 2.65 acetic acid. Degasser: ERC-3114; Ferryman sample: AS300, spectra Series thermoseparation products. Samples were dissolved in MeOH. Flow rate: 1.2 mL/min.

Extraction of (±)-Vasicinone 1

A total of 2.9 kg of P. harmala seeds-dried powder were extracted 3 times with methanol by stirring at 50°C for 1 hour. The extracts were combined and evaporated to dryness. The residue was dissolved in HCl solution (2%) and then filtered. The filtrate was extracted 2 times with pentane. The aqueous acid layer was basified (pH = 10) with NaOH and extracted 3 times with chloroform. The filtered extract was concentrated under reduced pressure and then the residue (35 g) was purified by chromatography on silica gel eluting successively with ethyl acetate-methanol (90:10 to 0:100) to afford 9 fractions; 1 g of (±)-vasicinone as a white solid was obtained after precipitation of fraction 2 in ethyl acetate-methanol (60:40). 9

N-Amino Acid Protection: Preparation of Compounds 2a-e

A total of 1 g of amino acid was dissolved in 15 mL of a solution of NaOH (2 M) followed by the addition of tosyl chloride (1 equiv.), trimethylamine (1 equiv.), and acetone (2 mL). After 10 minutes, the mixture was stirred at room temperature for 6 hours. The mixture was then extracted 3 times with diethyl ether after which the aqueous layer was acidified by the addition of concentrated HCl (pH = 1) and this aqueous phase was extracted 3 times with ethyl acetate. After evaporating the solvent, the residue was washed with ethyl acetate-diethyl ether (1:1) and the product was obtained after filtration. 11

Compound 2a: Tosylglycine

White solid. Yield 50%. 1H NMR (300 MHz, CDCl3) δH: 7.58 (2H, d, J = 8.1 Hz, H-2’ and H-6’), 7.33 (2H, d, J = 8.1 Hz, H-3’ and H-5’), 3.79 (2H, s, H-2), 2.43 (3H, s, CH3). 13C NMR (75 MHz, CDCl3) δC: 166.7 (C=O), 143.2 (C-1’), 136.0 (C-4’), 129.1 (C-3’ and C-5’), 126.7 (C-2’ and C-6’), 43.5 (C-2), 20.8 (CH3).

Compound 2b: Tosyl-L-Alanine

White solid. Yield 90%. 1H NMR (300 MHz, CDCl3) δH: 7.76 (2H, d, J = 7.8 Hz, H-2’ and H-6’), 7.35 (2H, d, J = 8.1 Hz, H-3’ and H-5’), 3.63 (2H, q, J = 6.9 Hz, H-2), 2.41 (3H, s, CH3), 1.31 (3H, d, J = 6.9 Hz, CH3(3)); 13C NMR (75 MHz, CDCl3) δC: 179.1 (C=O), 143.5 (C-1’), 139.1 (C-4’), 129.7 (C-3’’ and C-5’’), 127.0 (C-2’’ and C-6’’), 54.6 (C-2), 21.2 (CH3), 17.2 (C-3).

Compound 2c: Tosyl-L-Leucine

White solid. Yield 85%. 1H NMR (300 MHz, CDCl3) δH: 7.32 (2H, d, J = 8.4 Hz, H-2’ and H-6’), 7.80 (2H, d, J = 8.4 Hz, H-3’ and H-5’), 5.09 (1H, d, J = 3.6 Hz, NH), 3.96 (1H, dd, J = 6.3 Hz and J = 5.1 Hz, H-2), 2.44 (3H, s, CH3), 1.81 (2H, m, H-3), 1.54 (1H, dd, J = 6.9 Hz and J = 6.3 Hz, H-4), 0.92 (3H, d, J = 6.6 Hz, CH3(3)).
**Compound 2c: Tosyl-1-Phenylalanine**

White solid. Yield 40%. $^1$H NMR (300 MHz, CDCl$_3$) $\delta_{H}$: 7.58 (2H, d, $J = 8.1$ Hz, H-2′′ and H-6′′), 7.26 (2H, d, $J = 7.8$ Hz, H-3′′ and H-5′′), 7.15 (5H, m, H-2′, H-3′, H-4′, H-5′, and H-6′), 4.03 (1H, dd, $J = 8.4$ Hz and $J = 5.7$ Hz, H-2), 3.04 (1H, dd, $J = 13.8$ Hz and $J = 5.7$ Hz, H-3a), 2.87 (1H, $J = 13.8$ Hz and $J = 5.7$ Hz, H-2), 3.04 (1H, dd, $J = 8.7$ Hz, H-1), 5.47 (1H, d, $J = 7.8$ Hz and $J = 1.5$ Hz, H-7), 7.24 (2H, d, $J = 8.7$ Hz, H-3′′ and H-5′′), 5.91 (1H, dd, $J = 7.5$ Hz and $J = 5.7$ Hz, H-1), 5.47 (1H, d, $J = 8.1$ Hz, NH), 4.15 (3H, m, H-3a, H-3b and H-2), 2.59 (1H, m, H-2a), 2.37 (3H, s, CH$_3$), 2.05 (1H, m, H-2b), 1.46 (3H, m, $J = 6.9$ Hz, CH$_3$(3′′)), 1.3C NMR (75 MHz, CDCl$_3$) $\delta_{C}$: 173.1 (C=O), 143.0 (C-1′′), 137.7 (C-1′), 136.4 (C-4′′), 129.1 (C-3′′ and C-5′′), 129.0 (C-2′′ and C-6′′), 127.9 (C-2′′ and C-6′′), 126.6 (C-3′ and C-5′′), 126.3 (C-4′′), 57.4 (CH), 38.5 (CH$_3$), 20.3 (CH$_3$), 17.8 (2CH$_3$).

**Preparation of Esters 3a-e and 3b′-e′**

A mixture of (±)-vascione (50 mg, 0.24 mmol), N-protected amino acid (1 equiv), and APTS (p-TolueneSulfonic Acid) (0.075 equiv) was refluxed in 20 mL of anhydrous toluene using dean stark trap. The progress of the reaction was monitored by TLC. Once the reaction was completed, the residue was purified by column chromatography on silica gel and eluted with chloroform; the mixture of diastereoisomers was purified by preparative TLC to obtain the desired esters 3a-e and 3b′-e′.

**Compound 3a: 5-Oxo-2,3-Dihydropyrrolo[2,1-b]quinazolin-1-yl Tosyl-glycinamate**

White solid. Yield 70%. ES- HRMS $m/z$ [M+H]$^+$ 414.1401. $^1$H NMR (300 MHz, CDCl$_3$) $\delta_{H}$: 8.32 (1H, d, $J = 8.1$ Hz, H-6), 7.75 (2H, d, $J = 8.4$ Hz, H-3′ and H-5′), 7.52 (1H, m, H-8), 7.70 (1H, d, $J = 4.8$ Hz, H-9), 7.50 (1H, d, $J = 6.9$ Hz, H-7), 7.26 (2H, d, $J = 6.3$ Hz, H-3′′ and H-5′′), 6.01 (1H, d, $J = 7.8$ Hz and $J = 5.4$ Hz, H-1), 5.40 (1H, d, $J = 10.5$ Hz, NH), 4.24 (1H, m, H-3a), 4.11 (1H, m, H-3b), 3.95 (2H, d, $J = 5.4$ Hz, H-2′), 2.69 (1H, m, H-2a), 2.41 (3H, s, CH$_3$), 2.11 (1H, m, H-2b). $^1$C NMR (75 MHz, CDCl$_3$) $\delta_{C}$: 167.6 (C=O), 159.8 (C-5), 154.0 (C-10a), 148.3 (C-9a), 143.2 (C-1′′), 136.1 (C-4′′), 133.8 (C-8), 129.1 (C-3′′ and C-5′′), 127.1 (C-7), 126.8 (C-9), 126.7 (C-6′), 125.9 (C-2′′ and C-6′′), 120.6 (C-5a), 74.1 (C-1), 43.8 (C-3′), 43.1 (C-2′′), 26.6 (CH$_3$), 20.8 (C-2).
**Compound 3c**: 5-Oxo-2,3-Dihydropyrrolo[2,1-b]quinazolin-1-yl Tosyl-1-Lenticate

White solid. Yield 22%. ES- HRMS m/z [M+H]^+ 470.1749. 1H NMR (300 MHz, CDCl₃) δ: 8.35 (1H, dd, J = 8.1 Hz and J = 1.2 Hz, H-6), 7.75 (2H, d, J = 8.4 Hz, H-2” and H-6”), 7.70 (2H, m, H-8 and H-9), 7.55 (1H, td, J = 6.9 Hz and J = 1.5 Hz, H-7), 7.30 (2H, d, J = 8.7 Hz, H-3” and H-5”), 5.88 (1H, dd, J = 7.5 Hz and J = 5.1 Hz, H-1), 5.06 (1H, d, J = 10.2 Hz, NH), 4.19 (2H, m, H-3a and H-3b), 4.00 (1H, m, H-2), 2.54 (1H, m, H-2a), 2.42 (3H, CH₃), 2.05 (1H, m, H-2b), 1.85 (1H, m, H-4), 1.60 (2H, m, H-3’), 0.87 (3H, d, J = 6.6 Hz, CH₃), 0.85 (3H, d, J = 6.6 Hz, CH₃). 13C NMR (75 MHz, CDCl₃) δ C: 171.6 (C=O), 160.4 (C-5), 154.4 (C-10a), 149.1 (C-9a), 143.6 (C-1’), 137.1 (C-4’), 134.3 (C-8), 129.3 (C-3’), 127.7 (C-7), 127.5 (C-2” and C-6”), 127.2 (C-9), 126.4 (C-6), 121.3 (C-5a), 74.3 (C-1), 54.3 (C-2’), 43.5 (C-1’), 42.2 (CH₃), 29.6 (CH), 27.0 (C-2), 24.3 (CH₃), 22.6 (CH₃), 21.4 (CH₃).

**Compound 3d**: 5-Oxo-2,3-Dihydropyrrolo[2,1-b]quinazolin-1-yl (2S)-2-((4 Methylphenyl)sulfonamido)-2-Pheny lacetate

White solid. Yield 30%. ES- HRMS m/z [M+H]^+ 504.1589. 1H NMR (300 MHz, CDCl₃) δ: 8.34 (1H, dd, J = 8.1 Hz and J = 1.2 Hz, H-6), 7.79 (2H, m, H-8 and H-9), 7.66 (2H, d, J = 8.4 Hz, H-2’’ and H-6’’), 7.53 (1H, td, J = 6.9 Hz and J = 1.5 Hz, H-7), 7.24 (5H, m, H-2”, H-3”, H-4”, H-5’’ and H-6’’), 7.24 (2H, d, J = 8.1 Hz, H-3’’ and H-5”), 5.96 (1H, dd, J = 7.5 Hz and J = 5.1 Hz, H-1), 5.64 (1H, d, J = 7.8 Hz, NH), 5.21 (1H, d, J = 7.5 Hz, H-2), 4.05 (2H, m, H-3a and H-3b), 2.51 (1H, m, H-2a), 2.40 (3H, s, CH₃), 1.95 (1H, m, H-2b). 13C NMR (75 MHz, CDCl₃) δ C: 169.2 (C=O), 160.4 (C-5), 154.2 (C-10a), 148.0 (C-9), 143.5 (C-1’), 137.2 (C-4’), 134.9 (C-1’’), 134.3 (C-8), 129.5 (C-2’’ and C-6’’), 128.9 (C-3”’ and C-5”’), 128.8 (C-3” and C-5”), 127.2 (C-9), 127.3 (C-2” and C-6”), 127.2 (C-4”), 127.1 (C-5”), 126.4 (C-6’’), 121.2 (C-5a), 75.0 (C-1’’), 59.5 (C-2’’), 43.5 (C-3’’), 26.9 (C-2’), 21.3 (CH₃).

**Compound 3d’**: 5-Oxo-2,3-Dihydropyrrolo[2,1-b]quinazolin-1-yl (2S)-2-((4 Methylphenyl)sulfonamido)-2-Phenylacetate

White solid. Yield 32%. ES- HRMS m/z [M+H]^+ 490.1439. 1H NMR (300 MHz, CDCl₃) δ: 8.30 (1H, dd, J = 8.1 Hz and J = 1.2 Hz, H-6), 7.74 (3H, m, H-8, H-2’’ and H-6’’), 7.64 (1H, d, J = 8.1 Hz, H-9), 7.50 (1H, td, J = 8.1 Hz and J = 1.2 Hz, H-7), 7.30 (7H, m, H-2”, H-3”, H-4”, H-5’, H-6’, H-3’’, and H-5’’), 6.00 (1H, dd, J = 7.5 Hz and J = 5.1 Hz, H-1), 5.63 (1H, d, J = 8.4 Hz, NH), 5.14 (1H, d, J = 8.4 Hz, H-2), 4.16 (2H, m, H-3a and H-3b), 2.60 (1H, m, H-2a), 2.40 (3H, s, CH₃), 2.17 (1H, m, H-2b). 13C NMR (75 MHz, CDCl₃) δ C: 170.0 (C=O), 160.4 (C-5), 154.4 (C-10a), 148.9 (C-9a), 143.5 (C-1’’), 137.2 (C-4’’), 134.9 (C-1’’), 134.3 (C-8), 129.5 (C-2’’ and C-6’’), 128.9 (C-3’’ and C-5’’), 128.8 (C-3’’ and C-5’’), 127.2 (C-4’’), 127.1 (C-5’’), 126.4 (C-6’’), 121.2 (C-5a), 74.7 (C-1), 56.4 (C-2’’), 43.6 (C-3’’), 39.1 (C-3’’), 27.1 (C-2’), 21.3 (CH₃).

**Preparation of Compound 4**

Under argon atmosphere, (+)-vasicinone (100 mg) was dissolved in anhydrous dichloromethane (5 mL) and chloroacetic acid chloride (1 equiv.) was added in the presence of triethylamine (1 equiv.). The mixture was stirred for 24 hours at room temperature and then extracted with chloroform. The organic layer was dried over Na₂SO₄ after removal of solvent in vacuum and the resulting residue was purified by silica gel column chromatography (CHCl₃) to give compound 4. 

**Compound 3e**: 5-Oxo-2,3-Dihydropyrrolo[2,1-b]quinazolin-1-yl Tosyl-1-Phenylalaninat
**Preparation of Compounds 5a,b**

A mixture of compound 4 (0.1 g, 0.4 mmol) with an excess of trialkyl phosphite was stirred under reflux for 12 hours. The progress of the reaction was monitored by TLC and the excess trialkyl phosphite was evaporated in vacuum. The residue was purified by column chromatography on silica gel eluted with the mixture of EtOAc/CH3OH (95:5) to give the desired compounds 5a,b.14

**Compound 5a: 5-Oxo-2,3-Dihydropyrrole[2,1-b]quinazolin-1-yl 2-Chloroacetate**

Yellow oil. Yield 50%. ES- HRMS m/z [M+H]+ 353.0897. 1H NMR (300 MHz, CDCl3) δH: 8.33 (1H, d, J = 8.1 Hz , H-6), 7.73 (2H, m, H-8 and H-9), 7.52 (1H, m, H-7), 6.09 (1H, dd, J = 5.1 Hz, H-1), 4.29 (1H, m, H-3a), 4.12 (1H, m, H-3b), 3.81 (6H, d, J = 11.1 Hz, 2CH3), 3.11 (2H, d, J = 7.2 Hz, 2CH3). 13C NMR (75 MHz, CDCl3) δC: 166.3 (C-1 remaining), 160.5 (C-5), 159.8 (C-10a), 149.0 (C-9a), 134.2 (C-8), 127.5 (C-7), 126.5 (C-6), 125.9 (C-5b), 74.7 (C-1), 43.7 (C-3), 40.5 (C-2), 27.3 (C-2).

**Compound 5b: 5-Oxo-2,3-Dihydropyrrole[2,1-b]quinazolin-1-yl 2 (Dimethoxyphosphoryl)acetate**

Yellow oil. Yield 90%. ES- HRMS m/z [M+H]+ 381.1212. 1H NMR (300 MHz, CDCl3) δH: 8.33 (1H, d, J = 8.1 Hz , H-6), 7.75 (2H, m, H-8 and H-9), 7.51 (1H, m, H-7), 6.09 (1H, dd, J = 5.1 Hz, H-1), 4.25 (6H, m, H-3a, H-3b and 2(CH2-O)), 3.10 (2H, d, J = 4.8 Hz, H-1), 1.34 (6H, t, J = 7.2 Hz, 2CH3). 13C NMR (75 MHz, CDCl3) δC: 166.3 (O-C=O), 164.3 (C-5), 159.9 (C-10a), 148.4 (C-9a), 133.7 (C-8), 127.1 (C-7), 126.6 (C-6), 126.5 (C-5), 126.0 (C-5a), 74.1 (C-1), 63.5 (d, JPC = 9.9 Hz, OCH3), 43.7 (C-3), 35.2 (d, JPC = 13.8 Hz, CH2), 26.8 (C-2), 15.4 (CH3). 31P NMR (120 MHz, CDCl3): δp 21.2.

**Biological Evaluation**

**Cytotoxic assay.** (±)-Vasicinone 1 and the synthesized derivatives were tested for their cytotoxic activity toward 3 human cell lines: OVCAR-3, ovarian cancer cell line; MCF-7, a breast cancer cell line; and HCT-116, a human colon cancer cell line. Cells were distributed in 96-well plates at 3 × 10^3 cells/well in 100 µL. Each derivative was added to the culture medium at the concentration of 50 µg/mL and 100 µL of the mixture was added in each well. The cell growth can be controlled using the 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide which is a water-soluble tetrazolium salt with yellow coloration. The tested derivatives were resuspended in DMSO and then diluted in the buffer. Optical density was measured at 540 nm. Tamoxifen was used as a positive control and all measurements were performed in triplicate.15

**Acetylcholinesterase Inhibitor Activity Assay**

First, 25 µL of each compound was dissolved in DMSO and then diluted in the buffer (sodium phosphate buffer, pH = 8.0); 25 µL of enzyme solution, 50 µL of sodium phosphate, already prepared, and 125 µL of 5,5′-dithiobis[2-nitrobenzoic acid] were added in a 96-well microplate and then incubated for a period of 15 minutes at a temperature of 25°C. After the incubation step, 25 µL of a solution of acetylcholine iodide was added. The mixture was then incubated for 10 minutes at 25°C. Finally, at 412 nm, the absorbance was determined and the inhibitory power of the derivatives was given as a percentage of inhibition (I%) at different concentrations as follows:

\[
I(\%) = 100 - \left( \frac{A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

where Acontrol is the absorbance of the control reaction containing all reagents except the tested derivative and Asample is the absorbance of the test derivative.

For each compound, the IC50 was determined, which shows the concentration of each derivative through which it is capable of inhibiting 50% of the enzyme. Galanthamine was employed as positive control and the test was conducted in triplicate.16

**5-Lipoxygenase Inhibitor Activity Assay**

A volume of 20 µL of different concentrations of each compound was dissolved in DMSO and mixed individually with 150 µL of sodium phosphate buffer (pH 7.4) containing 20 µL of 5-LOX enzyme and 60 µL of linoleic acid (3.5 mM), yielding a final volume of 250 µL. All compounds were resuspended in DMSO following dilution in the buffer so that DMSO does not exceed 1%. The mixture was incubated at 25°C for 10 minutes and the absorbance was determined at 234 nm. Nordihydroguaiaretic acid was used as positive control. All measurements were performed in triplicate.17

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