

Drug Design, Synthesis, Characterization, In-silico Molecular Docking and In-vitro ACE-Inhibitory Evaluation of 1,3-thiazinan-4-one Derivatives as Potential Antihypertensive Agents

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Abstract: Hypertension is a leading modifiable risk factor for cardiovascular disease, and angiotensin-converting enzyme (ACE) is an established therapeutic target. This study reports the drug design, synthesis, characterization, in-silico docking and in-vitro ACE-inhibitory evaluation of 1,3-thiazinan-4-one derivatives (5a–5e). The compounds were prepared in 68–75% yield by a one-pot, solvent-free Staudinger/aza-Wittig sequence in which benzyl azide, triethyl phosphite, a substituted aromatic aldehyde and 3-mercaptopropionic acid were heated at 140–150 °C for 10–20 min. Purity was supported by single-spot TLC (R_f 0.45–0.65; hexane: ethyl acetate, 4:1) and melting points of 120–140 °C, and the products were characterized by FT-IR and ¹H NMR spectroscopy. In-silico analysis showed that four compounds satisfied all Lipinski criteria. Molecular docking against human ACE gave binding energies of –7.5 to –8.4 kcal/mol, with the thiophene derivative 5c (–8.4 kcal/mol) and the 4-nitrophenyl derivative 5d (–8.3 kcal/mol) ranking highest. In the in-vitro hippuryl-histidyl-leucine (HHL) assay, 5c and 5d inhibited ACE by 75.21 ± 4.25% and 71.48 ± 4.19% at 100 mg/mL, compared with 79.35 ± 4.84% for captopril; the IC₅₀ values recorded for 5c, 5d and captopril were 10.377, 20.431 and 5.808 mg/mL, respectively. These findings identify the 1,3-thiazinan-4-one scaffold, particularly 5c, as a promising starting point for ACE-directed antihypertensive.

Keywords: 1,3-thiazinan-4-one; Staudinger–aza-Wittig reaction; angiotensin-converting enzyme; molecular docking; antihypertensive; captopril.

INTRODUCTION

Hypertension is a chronic medical condition characterized by persistently elevated arterial blood pressure. Effective management and control of hypertension can significantly reduce the risk of cardiovascular complications, including stroke, ischaemic heart disease, and heart failure. Angiotensin-converting enzyme (ACE) converts angiotensin I to the potent vasoconstrictor angiotensin II and also degrades the vasodilator bradykinin; ACE inhibitors such as captopril therefore lower

blood pressure by suppressing angiotensin II formation and prolonging bradykinin action. [1,2] Heterocyclic scaffolds underpin a large proportion of drugs and natural products. Among them, 1,3-thiazinan-4-ones, six-membered rings containing an N–C–S linkage, have been reported to show cyclooxygenase-2 inhibitory, antitumor, antimalarial, antifungal, antibacterial and antioxidant activities. [3,4] Their cardiovascular potential has received comparatively little attention.

Several routes to thiazinanones have been described, including conventional reflux, cyclization of Schiff bases with mercapto acids and ultrasound-assisted one-pot condensation. [5-7] The Staudinger/aza-Wittig strategy, in which an organic azide reacts with a phosphorus (III) reagent to give an iminophosphorane that condenses with a carbonyl compound, offers a solvent-free alternative. [8–10] Its main drawback with triphenylphosphine is the water-insoluble triphenylphosphine oxide by-product, which usually requires chromatography; replacing triphenylphosphine with triethyl phosphite gives water-soluble triethyl phosphate instead. Computational tools now allow compounds to be prioritized before synthesis. Molecular docking predicts binding orientation and affinity within a target site, [11,12] and drug-likeness filters such as Lipinski's rule of five and web tools such as SWISS-ADME help to screen for oral bioavailability [13,14]. Docking against ACE has been used to evaluate natural products and designed heterocycles, [15,16] ACE inhibition is commonly confirmed in vitro by measuring hippuric acid released from hippuryl-histidyl-leucine (HHL). [17–19] In the present work, 1,3-thiazinan-4-ones were designed, synthesized by a solvent-free triethyl phosphite-mediated Staudinger/aza-Wittig route, characterized spectroscopically, docked against ACE and evaluated for in-vitro ACE inhibition using captopril as the reference.

2. Materials and Methods

2.1 Materials

Benzyl bromide, sodium azide, acetone, dichloromethane, anhydrous sodium sulphate, sodium bicarbonate, triethyl phosphite, 3-mercaptopropionic acid and the aldehydes (4-methoxy benzaldehyde, 4-isopropylbenzaldehyde, thiophene-2-carboxaldehyde, 4-nitrobenzaldehyde and 4-methylbenzaldehyde) were used as supplied. For the enzyme assay, ACE, hippuryl-histidyl-leucine (HHL), sodium borate buffer, sodium chloride, ethyl acetate, hydrochloric acid and captopril were used. Melting points were determined with a digital melting-point apparatus. TLC was run on pre-coated silica gel 60 GF254 plates (Merck). Absorbance was measured with a UV spectrophotometer.

2.2 Molecular design and in-silico drug-likeness

Structures were drawn with Chem Sketch and energy-minimized with Chem3D Ultra. LogP, molecular weight, hydrogen-bond donors and acceptors and rotatable bonds were calculated with Molinspiration and SwissADME, and molar refractivity with Chem Sketch. Drug-likeness was judged against Lipinski's criteria (molecular weight < 500, LogP < 5, hydrogen-bond donors < 5, hydrogen-bond acceptors < 10, molar refractivity < 150). [13] Toxicity was predicted with ADMETlab 3.0.

2.3 Molecular docking

The crystal structure of human ACE was retrieved from the RCSB Protein Data Bank (PDB ID 1O86). Minimized ligands were docked with PyRx (Auto-Dock Vina), and the binding energy (kcal/mol) of the best pose was recorded; two-dimensional interaction maps were generated for the highest-ranked compounds.

2.4 Synthesis

Benzyl azide was added to a stirred solution of benzyl bromide in a water/acetone mixture and stirred at room temperature for 24 h. The mixture was extracted with dichloromethane, and the combined organic layers were dried over anhydrous sodium sulphate and concentrated under reduced pressure to give benzyl azide (99%), used without further purification. General procedure for 1,3-thiazinan-4-ones (5a–e). Benzyl azide (1.22 mL) was added dropwise to triethyl phosphite (1.72 mL) with stirring until nitrogen evolution ceased. The aldehyde was then added (4-methoxybenzaldehyde 1.36g; 4-isopropylbenzaldehyde 1.52 mL; thiophene-2-carboxaldehyde 1.0 mL; 4-nitrobenzaldehyde 1.51 g; 4-methylbenzaldehyde 1.20 g; approximately 10 mmol scale), followed by dropwise addition of 3-mercaptopropionic acid (1.06 mL). The solvent-free mixture was heated at 140–150 °C for 10–20 min (Figure 1). After completion (TLC), excess acid was neutralized with aqueous sodium bicarbonate, and the product was extracted into dichloromethane, dried over anhydrous sodium sulphate, concentrated under reduced pressure and recrystallized from ethanol.

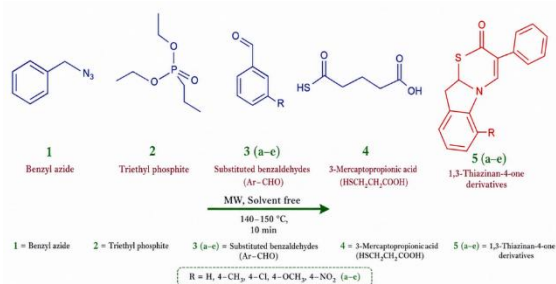


Figure 1. Synthetic scheme for -1,3-thiazinan-4-ones 5a-e (R = 4-OCH₃, 4-CH(CH₃)₂, thiophen-2-yl, 4-NO₂, 4-CH₃).

2.5 Purity and spectral characterization

Reaction progress and purity were monitored by TLC (hexane: ethyl acetate, 4:1), and a single spot was taken as evidence of purity. FT-IR and ¹H NMR spectra of the purified compounds were recorded, and the observed bands were compared with standard group-frequency ranges.

2.6 In-vitro ACE-inhibition assay

ACE inhibition was determined from the amount of hippuric acid released from HHL. ACE solution (50 µL, 25 mU/mL) was incubated with 50 µL of test solution (compounds 5c and 5d, or captopril, at 25, 50 and 100 mg/mL) at 37 °C for 10 min. Substrate solution (150 µL; 8.3 mM HHL in 50 mM sodium borate buffer containing 0.5 M NaCl, pH 8.3) was added and incubation continued for 30 min at 37 °C. The reaction was stopped with 250 µL of 1 M HCl, hippuric acid was extracted with 0.5 mL ethyl acetate and centrifuged (800 × g, 15 min), and 0.2 mL of the upper layer was evaporated under vacuum, redissolved in 1 mL distilled water and read at 228 nm. Percentage inhibition was calculated as 100 – [T/C] × 100, where T and C are the absorbances of the test and control (HHL only) reactions. IC₅₀ was derived by linear regression of percentage inhibition against concentration. Measurements were made in triplicate and are given as mean ± standard deviation (SD). [17–19]

3. Results

3.1 Chemistry and TLC

The Staudinger/aza-Wittig sequence proceeds through formation of an iminophosphorane from benzyl azide and triethyl phosphite with loss of nitrogen, aza-Wittig condensation with the aldehyde to give an imine, addition of the thiol of 3-mercaptopropionic acid to the imine carbon, and

intramolecular amide formation with loss of water to close the six-membered ring. All five compounds were obtained as white to pale-yellow crystalline solids in 68–75% yield, soluble in dichloromethane, dimethylformamide and dimethyl sulfoxide (Table 1). Each gave a single TLC spot (Figure 2), with R_f values between 0.45 and 0.65 that decreased with increasing polarity of the aryl substituent.

Table 1. Physical data of 1,3-thiazinan-4-ones 5a-e.

Code	Aryl (Ar)	Formula	Yield (%)	M.P (°C)	R _f
5a	4-Methoxyphenyl	C ₁₈ H ₁₉ NO ₂ S	75	130	0.65
5b	4-Isopropylphenyl	C ₂₀ H ₂₃ NOS	74	125	0.60
5c	Thiophen-2-yl	C ₁₂ H ₁₁ NOS ₂	72	135	0.50
5d	4-Nitrophenyl	C ₁₇ H ₁₆ N ₂ O ₃ S	68	140	0.45
5e	4-Methylphenyl (p-tolyl)	C ₁₆ H ₁₉ NOS	73	120	0.47

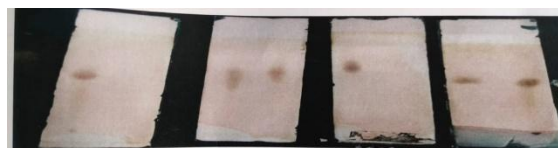


Figure 2. TLC of the synthesized compounds (hexane: ethyl acetate, 4:1), showing a single spot for each.

3.2 FT-IR spectroscopy

The spectra showed aromatic and aliphatic C–H stretching bands at 3084–2927 cm⁻¹, C=C stretching at 1577–1659 cm⁻¹ and C–N stretching at 1251–1275 cm⁻¹ for the series. Substituent-specific bands were seen for 5a (C–O, 1049 cm⁻¹), 5b (isopropyl methyl, 1383 cm⁻¹), 5c (thiophene-ring band, 800 cm⁻¹) and 5e (methyl C–H, 2868 cm⁻¹). Para-disubstituted ring bands at 835–881 cm⁻¹ and monosubstituted (benzyl) ring bands at 671–700 cm⁻¹ were present for the aryl-substituted members. ¹H NMR spectra were recorded for all five compounds to confirm the substituted thiazinanone framework.

3.3 In-silico drug-likeness and toxicity

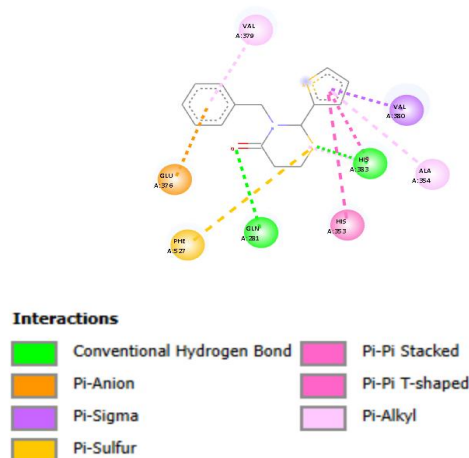
Five compounds (5a, 5b, 5c, 5d, 5e) satisfied all Lipinski criteria, and No compound had hydrogen-bond donors, and all had molecular weights of 289–328. 5c had the lowest LogP (2.62), indicating the most hydrophilic character of the series. The ADMETlab 3.0 toxicity predictions did not flag a toxicity risk for the compounds.

Table 2. Drug-likeness parameters (M.W.; M.R., cm³/mol; HBA/HBD, H-bond acceptors/donors) and ACE docking scores (kcal/mol) of 5a-e.

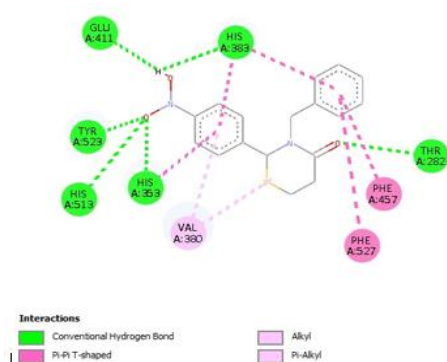
Code	M.W.	M.R.	HBA	HBD	LogP	Dock. score
5a	313.41	91.69	3	0	4.75	-7.6
5b	325.47	99.44	2	0	4.18	-7.9
5c	289.42	84.87	2	0	2.62	-8.4
5d	328.39	91.64	4	0	4.37	-8.3
5e	297.41	90.16	2	0	4.64	-7.5

3.4 Molecular docking with ACE INHIBITOR

All five compounds docked favorably into ACE with binding energies from -7.5 to -8.4 kcal/mol (Table 2). 5c (-8.4) and 5d (-8.3) ranked highest. In the 5c pose (Figure 3a) the carbonyl oxygen and Thiazinan sulphur formed conventional hydrogen bonds with Gln281 and His383, the thiophene ring made π - π contact with His353, π - σ contact with Val380 and π -alkyl contacts with Ala354, the benzyl ring made a π -anion contact with Glu376, and Phe527 contributed a π -sulphur interaction. In the 5d pose (Figure 3b) the nitro group formed hydrogen bonds with Glu411, His383, Tyr523, His513 and His353, the lactam carbonyl hydrogen-bonded to Thr282, and Phe457 and Phe527 made π - π T-shaped contacts, with additional alkyl/ π -alkyl contact from Val380.



(a) Compound 5c



(b) Compound 5d

Figure 3. Two-dimensional interaction maps of (a) 5c and (b) 5d in the active site of ACE.

3.5 In-vitro ACE inhibition

Compounds 5c and 5d, selected on the basis of their docking scores, inhibited ACE in a concentration-dependent manner (Table 3, Figure 4). At 100 mg/mL, 5c and 5d produced $75.21 \pm 4.25\%$ and $71.48 \pm 4.19\%$ inhibition, respectively, compared with $79.35 \pm 4.84\%$ for captopril. The potency order was captopril > 5c > 5d at every concentration, and the IC₅₀ values recorded were 5.808, 10.377 and 20.431 mg/mL for captopril, 5c and 5d, respectively.

Table 3. ACE inhibition (% , mean $\pm$ SD, n = 3) by 5c, 5d and captopril at 25, 50 and 100 mg/mL; IC₅₀ in mg/ml.

Sample	25	50	100	IC ₅₀
Captopril	19.81 $\pm$ 3.32	40.78 $\pm$ 4.55	79.35 $\pm$ 4.84	5.808
5c	15.89 $\pm$ 2.04	37.21 $\pm$ 4.40	75.21 $\pm$ 4.25	10.377
5d	13.87 $\pm$ 2.52	32.90 $\pm$ 3.60	71.48 $\pm$ 4.19	20.431

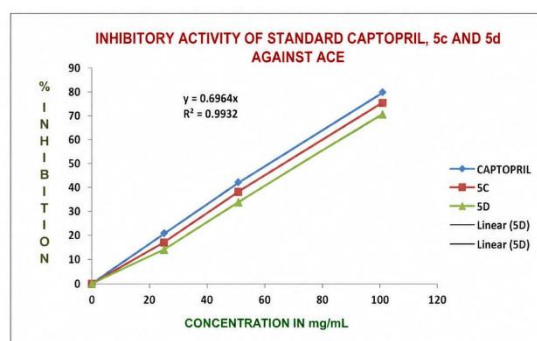


Figure 4. Concentration-dependent inhibition of ACE by captopril, 5c and 5d.

4. Discussion

The solvent-free triethyl phosphite-mediated route gave all five thiazinanones in 68–75% yield within 10–20 min without chromatographic removal of a phosphine oxide, supporting the practical advantage of replacing triphenylphosphine. The yield decreased slightly for the electron-poor 4-nitrophenyl derivative (68%) relative to the electron-rich and neutral analogues (72–75%). The docking ranking agreed with the enzyme assay: the two highest-scoring compounds, 5c and 5d, were also the two tested in vitro and both showed substantial inhibition, with 5c more active than 5d and approaching captopril at the highest concentration. In the docked poses, His353, His383 and Glu411 are residues generally associated with the catalytic zinc-binding region of ACE, and Gln281 and

His513 with the S2' subsite; contacts with these residues are consistent with binding at the catalytic site, as seen with other heterocyclic and natural-product ACE ligands.[15,16,20] The multiple hydrogen bonds of the nitro group of 5d did not translate into higher activity than the thiophene analogue 5c, which suggests that hydrophobic and π -contacts, and the low LogP of 5c, also contribute. Several limitations should be noted. Only two compounds were tested in vitro, at three concentrations expressed in mg/mL, which is high for an enzyme assay, and the IC50 values recorded for the three samples do not agree with the percent-inhibition data in Table 3 (for example, captopril inhibited only 40.78% at 50 mg/mL), so the dose-response should be repeated over a wider range, preferably in μ M units, before potency is compared with the clinical standard. Docking scores are predictions rather than affinities, 5b violates one Lipinski criterion, and the toxicity assessment was computational only. A larger series with varied electron-donating and electron-withdrawing substituents, enzyme-kinetic studies and in-vivo antihypertensive testing are needed to establish a structure-activity relationship.

5. Conclusions

Five 1,3-thiazinan-4-one derivatives were synthesized in good yield by a rapid solvent-free Staudinger/aza-Wittig route and characterized by TLC, melting point and FT-IR/¹H NMR spectroscopy. All were predicted to be drug-like, and docking identified the thiophene derivative 5c and the 4-nitrophenyl derivative 5d as the best ACE binders. In the HHL assay, both inhibited ACE in a concentration-dependent manner, reaching 75.21% and 71.48% inhibition at 100 mg/mL against 79.35% for captopril. The 1,3-thiazinan-4-one scaffold, particularly 5c, is therefore a promising lead for further antihypertensive optimization, subject to repeat dose-response work and in-vivo validation.

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Author Contributions: Vigneshwaran K: conceptualization, drug design, synthesis, characterization, molecular docking, in-vitro ACE-inhibition assay, data analysis and writing—original draft. Ramesh Kumar S: supervision, project administration, validation and critical review. Rekha S, Iswarya M, Aravindh Rao s: editing of the manuscript. All authors contributed to the interpretation of the results, reviewed the manuscript critically, and approved the final version.

Conflicts of Interest: The authors declare no conflict of interest.

References

- [1]. Nelson EB, Pool JL, Taylor AA. Pharmacology of angiotensin converting enzyme inhibitors. A review. *Am J Med.* 1986;81(4C):13-18.
- [2]. Regulski M, Regulska K, Stanis B, et al. Chemistry and pharmacology of angiotensin-converting enzyme inhibitors. *Curr Pharm Des.* 2015;21(13):1764-1775.
- [3]. Mansuroglu DS, et al. Chemistry of substituted thiazinanes and their derivatives. *Molecules.* 2020;25(23):5610.
- [4]. Bosenbecker J, Barden VD, Difabio R, et al. Synthesis and in vitro antioxidant evaluation of six-membered thiazinones. *J Biochem Mol Toxicol.* 2014;28(9):425-432. doi:10.1002/jbt.21581.
- [5]. Mohamed SK, Abdel Hamid AA, Omara W, Jaber AM, Albayabi M. *J Chem Pharm Res.* 2013;5(1):19-31.
- [6]. Al-Hiti WF, Saeed MA. BEST: *Int J Humanit Arts Med Sci.* 2017;5:65-74.
- [7]. Gouvea DP, Berwaldt GA, Neuenfeldt PD, Nunes RJ, Almeida WP, Cunico W. *J Braz Chem Soc.* 2016;27:1109-1115.
- [8]. Sathishkumar M, Shanmugavelan P, Nagarajan S, Maheswari M, Dinesh M, Ponnusamy A. *Tetrahedron Lett.* 2011;52:2830.
- [9]. Palanikumar K, Sathishkumar M, Mariappan A, Archana S, Ponnusamy A. *J Iran Chem Soc.* 2011.
- [10]. Nagarajan S, Shanmugavelan P, Dinesh M, Ponnusamy A. *J Braz Chem Soc.* 2011;22:2065.
- [11]. Ferreira LG, dos Santos RN, Oliva G, Andricopulo AD. Molecular docking and structure-based drug design strategies. *Molecules.* 2015;20(7):13384-13421.

- [12]. Jakhar R, Dangi M, Khichi A, Chhillar AK. Relevance of molecular docking studies in drug designing. *Curr Bioinform.* 2020;15(4):270-278.
- [13]. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev.* 1997;23:3-25.
- [14]. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci Rep.* 2017;7:42717. doi:10.1038/srep42717.
- [15]. Muhammad SA, Fatima N. In silico analysis and molecular docking studies of potential angiotensin-converting enzyme inhibitor using quercetin glycosides. *Pharmacogn Mag.* 2015;11(42).
- [16]. Maheswari C, Venkatnarayanan R, Manavalan R, Johnson JS, Subadradevi J, Babu P. *Scholars Research Library.* 2015;7(8):53-63.
- [17]. Rachmawati H, Soraya IS, Kurniati NF, Rahma A. In vitro study on antihypertensive and antihypercholesterolemic effects of a curcumin nanoemulsion. *Sci Pharm.* 2016;84:131-140.
- [18]. Zhuang X-D, Liao L-Z, Dong X-B, et al. *Drug Des Devel Ther.* 2016;10:129-139.
- [19]. Li G-H, Qu M-R, Wan J-Z, You J-M. Antihypertensive effect of rice protein hydrolysate with in vitro angiotensin I-converting enzyme inhibitory activity in spontaneously hypertensive rats. *Asia Pac J Clin Nutr.* 2007;16(Suppl 1):275-280.
- [20]. Vázquez-Valadez VH, Abrego VH, Martínez PA, Torres G, Zúñiga O, Escutia D. *Open Med Chem J.* 2013;7:30-38.