



ARAŞTIRMA / RESEARCH

Synthesis of thiazole-phenylacetic acid compounds as dual antibacterial-COX enzymes inhibitors

Tiyazol-fenilasetik asit bileşiklerinin dual antibakteriyel-COX enzim inhibitörleri olarak sentezleri

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Abstract

Purpose: Present a new potential dual drug for patient suffering both inflammation and infection was aim of this study therefore investigation of anti-inflammatory and antimicrobial activity of new thiazole-phenylacetic acid compounds derivatives as a potential dual drug was carried out.

Materials and Methods: Anti-inflammatory activity of new synthesized drugs were determined via fluorometric assay. Assay based on fluorometric detection of intermediates prostaglandin G1 and G2 during COX1 (cyclooxygenases-1) and COX2 (cyclooxygenases-2) enzyme inhibition, respectively. Ibuprofen and Nimesulide were used as reference drugs. Antimicrobial activity of compounds in parallel were evaluated with microbroth dilution assay. Two-fold serial dilution of compounds were tested against microorganisms and minimum inhibition concentration of compound were determined according to CLSI (Clinical and Laboratory Standards Institute). Chloramphenicol was used as a control drug.

Results: Fluorometric assay and antimicrobial assay results were compared. The observation was compound 2b had important inhibitory activity on COX1 and COX2 but activity of 2d carrying 4-chlorophenyl and phenyl was more successful than 2b and also antimicrobial activity of 2d against microorganism was better or same as reference drug chloramphenicol.

Conclusion: Between the new synthesized compound, 2d exhibited remarkable anti-inflammatory and antibacterial activity. 2d can be evaluated as a promising drug potential. Further investigation on scaffold of 2d will help to develop new alternatives for dual anti-inflammatory and antibacterial agent which can provide relief for patients in suffering from the symptoms of these diseases.

Key words: Inflammation, infection, cyclooxygenases, antibacterial.

Öz

Amaç: Bu çalışmanın amacı inflamasyon ve enfeksiyon geçiren hastalara önerilebilecek çift etkili potansiyel ilaçlar sunmak olup bu bağlamda tiyazol fenilasetik asit türevlerinin antiinflamatuvar ve antibakteriyel aktiviteleri araştırmaktır.

Gereç ve Yöntem: Yeni sentezlenen bu bileşiklerin antiinflamatuvar etkileri fluorometrik yöntem ile belirlenmiştir. Bu yöntem COX1 (siklooksijenaz 1) ve COX2 (siklooksijenaz-2) enzim inhibisyonu sırasında oluşan ara ürünler prostaglandin-1 ve prostaglandin-2'nin belirlenmesine dayanmaktadır. Ibuprofen ve Nimesulid referans ilaç olarak kullanılmıştır. Antimikrobiyal aktiviteyi belirlemek üzere mikrobroth dilüsyon testi kullanılmıştır. Bileşiklerin iki kat seri dilüsyonları mikroorganizmalara karşı denenmiş ve bileşiklerin minimum inhibisyon konsantrasyonları CLSI'ye göre belirlenmiştir. Kloromfenikol kontrol ilaç olarak kullanılmıştır.

Bulgular: Fluorometrik yöntem ve antimikrobiyal aktivite sonuçları karşılaştırılmıştır. 2b nin önemli COX1 ve COX2 inhibisyon etkisi olduğu gözlenmiş olup, 4-klorofenil ve fenil grubu taşıyan 2d nin ise aktivitesinin daha iyi olduğu gözlenmiştir. Aynı sırada 2d nin antimikrobiyal etkisinin kontrol ilaç olarak kullanılan kloromfenikole eş ya da daha iyi olduğu belirlenmiştir.

Sonuç: Sentezlenen bileşikler arasında 2d hem antiinflamatuvar hemde antibakteriyel aktivite göstermiştir. 2d umut veren potansiyel bir ilaç olarak değerlendirilebilir. 2d'nin iskelet yapısı üzerine yapılacak olan ileri çalışmalar yeni, alternatif dual antiinflamatuvar ve antibakteriyel ilaç gelişimine ve böylece her iki hastalığın semptomlarından muzdarip hastalar için umut olacaktır.

Anahtar kelimeler: Inflamasyon, enfeksiyon, siklooksijenaz, antibakteriyel.

INTRODUCTION

Inflammation is the immune system's response to infection and a variety of injury. It has been associated with the pathogenesis of some disorders as arthritis¹, cancer², neurodegenerative³ and cardiovascular diseases⁴. The acute stage of inflammation is inevitable and considered by quick influx of blood granulocytes, followed rapidly by monocytes that settled into inflammatory macrophages which subsequently proliferate and affect the functions of resident tissue macrophages. This progression causes the cardinal signs of acute inflammation^{5,6}. After remove of initiating noxious stimulus through phagocytosis, the inflammatory reaction reduces and macrophages and lymphocytes return to normal pre-inflammatory numbers and phenotypes. High resolution and reparation of the tissue damage are normal results of the acute inflammatory process⁷.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most prescribed agents for the treatment of various inflammatory diseases⁸. The mechanism of action of NSAIDs was elucidated with the identification of cyclooxygenases (COX) enzyme in 1971 and has been understood that inhibition of COXs play important role on the repression of prostaglandin biosynthesis from arachidonic acid. There are two isoforms of COXs; COX-1 and COX-2⁹. The constitutive COX-1 isoform is produced in the most tissues and is responsible for the synthesis of cytoprotective prostaglandins (PGs) in the gastrointestinal system, vascular homeostasis and platelet aggregation, whereas the inducible COX-2 is expressed in some tissues to produce PGs and thus initializes the inflammation¹⁰.

Multidrug therapy is of importance in case of infection and inflammation occur together in the patient especially who suffering from damaged liver or kidney functions, patients with diseases of the gastrointestinal system¹¹. As a new insight combine drugs such as COX inhibitory-antibacterial agent has attracted attention for treatment of such patients¹²⁻¹⁶.

Thiazole is an important heterocyclic ring which is often subjected to new drug development studies. There are some reports including COX enzymes inhibitory and antibacterial effects of thiazole based compounds. Aryl acetic acid derivatives such as indomethacin, sulindac, etodolac, ketorolac, and diclofenac constitute a class of nonsteroidal anti-

inflammatory agents, which are inhibitors of COX enzymes¹⁷. Thus, in this study, some thiazole based phenyl acetic acid derivatives were synthesized to investigate their possible effects as dual COX inhibitory-antibacterial agents.

MATERIALS AND METHODS

The chemicals used in the syntheses and biological activity studies were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). ¹H NMR spectra was recorded by a Bruker 300 MHz digital FT-NMR spectrometer (Bruker Bioscience, Billerica, MA, USA) in DMSO-d₆. HRMS studies were performed on Shimadzu LCMS-IT-TOF system (Shimadzu, Tokyo, Japan).

Preparation of 4-thioureido-phenylacetic acid (1)

4-Aminophenylacetic acid (30 mmol, 4,53 g), HCl (37%, 33 mmol, 3,26 mL), and NSCN (33 mmol, 2,52 g) were refluxed in H₂O (5 mL) for 2h. The reaction mixture was poured into iced-water, precipitated product was washed with water, dried, and recrystallized from ethanol.

General Synthesis procedure for 1-[4-[4-[2-(dimethylamino)ethyl]-piperazine-1-yl]phenyl]-3-(4-substitutedphenyl)prop-2-en-1-one derivatives (2a-2d)

The compound 1 (1 mmol, 0,21g) and 2-bromoacetophenone or 2-chloro-1,2-diaryl-ethanone derivative (1 mmol) in EtOH (10 mL) was refluxed for 6 h. The resulting solid was filtered, washed with water, dried, and recrystallized from ethanol.

COX-1 and COX-2 inhibitory activity

Inhibitory potency of the compounds (2a-2d) against COX-1 and COX-2 enzymes was determined by using fluorometric COX-1 and COX-2 inhibitor screening kits (Biovision, Switzerland). Experimental protocol was followed as described in the guides of the supplier^{18,19}. All of the pipetting in the assay were performed by Biotek Precision robotic system (BioTek Instruments, Inc., Winooski, VT, USA). Fluorescence (Ex/Em = 535/587 nm) of the samples were kinetically

measured by BioTek-Synergy H1 multimode microplate reader (BioTek Instruments, Inc., Winooski, VT, USA) at 25°C for 5-10 min. Appropriate two points (T1 and T2) in the linear range of the plot were chosen, and the corresponding fluorescence values (RFU1 and RFU2) were obtained. Enzymatic assay was applied in the concentration range of 10⁻³-10⁻⁶ M for all compounds. Ibuprofen and Nimesulide were used as control agents.

Antimicrobial activity

Microbiological studies were performed according to following guides: CLSI reference M07-A9 broth microdilution method20 for bacterial strains. Synthesized compounds were tested for their in vitro growth inhibitory activity against *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (ATCC 1911), *Klebsiella pneumoniae* (NCTC 9633), *Escherichia coli* (ATCC 35218) and *Escherichia coli* (ATCC 25922). Chloramphenicol was used as a control drug. The cultures were obtained from Mueller–Hinton broth (Difco) for the bacterial strains after overnight incubation at 37 °C. The inocula of test microorganisms adjusted to match the turbidity of a Mac Farland 0.5 standard tube as determined with a spectrophotometer and the final inoculum size was 5x10⁵ CFU/mL for antibacterial assay. Testing was carried out in Mueller–Hinton broth and the two-fold serial dilutions technique was applied. The last

well on the microplates containing only inoculated broth was kept as controls and the last well with no growth of microorganism was recorded to represent the MIC expressed in µg/mL. The compounds were dissolved in DMSO and further dilutions of compounds and standard drugs in test medium were prepared at the required quantities of 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 1.95 µg/mL concentrations with Mueller–Hinton broth. The completed plates were incubated for 24h. At the end of the incubation, resazurin (20 µg/mL) was added into each well and plates were incubated for 2h. MIC50 values were determined using a microplate reader at 590 nm excitation, 560 nm emission.

Statistical analysis

In enzymatic assay, each concentration was analysed in quadruplicate. The slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU2 – RFU1) values by the time ΔT (T2 – T1) were calculated by using following equation:

$$\% \text{ Relative inhibition} = (\text{Slope of EC} - \text{Slope of S} / \text{Slope of EC}) \times 100$$

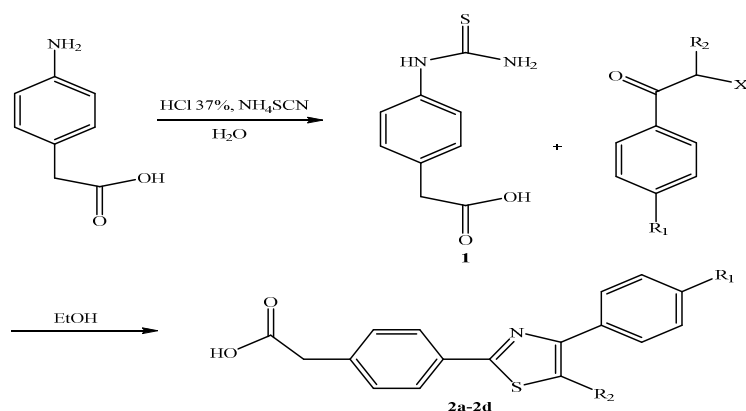
Both sample and enzyme control values are corrected with blank-reading value. Data were expressed as mean ± standard deviation (SD). The IC₅₀ value was calculated from the plots of enzyme activity against concentrations by applying regression analyses on Microsoft Excel 2013.

Table 1. Inhibitory activity and selectivity of the compounds (2a-2d) towards COX-1 and COX-2

Compound	COX-1 Inhibition %				IC ₅₀ (µM)	COX-2 Inhibition %				IC ₅₀ (µM)	SI*
	10 ⁻³ M	10 ⁻³ M	10 ⁻³ M	10 ⁻⁴ M		10 ⁻⁵ M	10 ⁻³ M	10 ⁻⁴ M	10 ⁻⁵ M		
2a	66.65 ±3.33	50.27 ±2.5	36.42 ±1.82	24.65 ±1.23	78.25 ±3.91	67.29 ±3.36	51.34 ±2.56	39.27 ±1.96	28.41 ±1.42	58.33 ±2.92	1.34
2b	73.46 ±3.67	56.27 ±2.81	44.27 ±2.21	36.70 ±1.84	19.10 ±0.95	72.39 ±3.61	55.84 ±2.79	46.38 ±2.32	38.39 ±1.92	16.16 ±0.81	1.18
2c	69.27 ±3.46	52.21 ±2.61	39.27 ±1.96	28.27 ±1.41	50.34 ±2.5	68.29 ±3.41	59.65 ±2.98	38.51 ±1.92	28.16 ±1.41	39.37 ±1.97	1.28
2d	76.34 ±3.81	58.15 ±2.90	48.43 ±2.42	34.75 ±1.73	14.84 ±0.74	75.57 ±3.77	59.34 ±2.96	47.51 ±2.37	32.63 ±1.63	17.08 ±0.85	0.87
Ibuprofen	80.35 ±4.017	65.72 ±3.28	53.45 ±2.67	44.71 ±2.23	3.71 ±0.18	81.28 ±4.06	62.02 ±3.10	54.29 ±2.71	46.27 ±2.31	3.66 ±0.18	1.01
Nimesulide	79.03 ±3.95	61.21 ±3.06	52.67 ±2.63	41.25 ±2.06	5.52 ±0.27	84.74 ±4.23	71.74 ±3.58	60.47 ±3.02	42.99 ±2.15	1.16 ±0.06	4.76

SI: selectivity index IC₅₀: Half maximal inhibitory concentration;

*The selectivity index (SI) was calculated as IC₅₀ (COX-1)/IC₅₀ (COX-2)



Scheme 1: Synthesis of 4-[4-(4-Substitutedphenyl)-5-substitutedthiazol-2-yl]phenylacetic acid derivatives (2a-2d). Compound 2a: R₁: -H, R₂: -H; Compound 2b: R₁: -Cl, R₂: -H; Compound 2c: R₁: -H, R₂: -Ph; Compound 2d: R₁: -Cl, R₂: -Ph

RESULTS

In this work, four 4-[4-(4-Substitutedphenyl)-5-substitutedthiazol-2-yl]phenylacetic acid derivatives (2a-2d) were prepared as presented in the Scheme 1. Structure elucidation of synthesized compounds were carried out by spectroscopic analyses.

The COX-1 and COX-2 inhibitory activity of compounds 2a-2d were evaluated by a Fluorimetric method^{18,19} (Table 1). In the series, compound 2d, carrying 4-chlorophenyl and phenyl variable groups at 4th and 5th positions of thiazole, indicated promising COX-1 and COX-2 inhibitory activity. This compound displayed nonselective COX inhibitory effect with an IC₅₀ values of 14.84 μM and 17.08 μM against COX-1 and COX-2 enzymes,

respectively. Compound 2b also showed a good inhibitory activity against both COX isoforms. IC₅₀ values of 19.10 μM and 16.16 μM against COX-1 and COX-2 enzymes were recorded for compound 2b. Reference agents ibuprofen and nimesulide displayed nonselective COX inhibition and selective COX-2 inhibition, respectively as expected (Table 1).

Antibacterial activity of test compounds were determined by microbroth inhibition assay (MIC). As a result, almost all compounds were effective on microorganism especially compound 2d showed good antimicrobial activity as much as reference drug 2d was more effective on *L. monocytogenes* and *K. pneumoniae* than reference drug. The other compounds are comparable with the reference but not better.

Table 2. Antibacterial activity (MIC μg/mL) of compounds 2a-2d against pathogenic bacteria.

Compound	<i>Sa</i>	<i>Ef</i>	<i>Lm</i>	<i>Kp</i>	<i>Ec-1</i>	<i>Ec-2</i>
2a	12.5	12.5	12.5	6.25	6.25	12.5
2b	12.5	12.5	12.5	6.25	6.25	12.5
2c	12.5	12.5	12.5	6.25	6.25	12.5
2d	6.25	6.25	6.25	6.25	6.25	6.25
Chloramphenicol	6.25	6.25	12.5	12.5	6.25	6.25

Sa: *Staphylococcus aureus* (ATCC 25923). Ef: *Enterococcus faecalis* (ATCC 29212). Lm: *Listeria monocytogenes* (ATCC 1911). Kp: *Klebsiella pneumoniae* (NCTC 9633). Ec-1: *Escherichia coli* (ATCC 35218). Ec-2: *Escherichia coli* (ATCC 25922).

DISCUSSION

Enzyme inhibition studies exposed that synthesized compounds were less active than reference drugs against both COX isoforms. Besides, it was determined that synthesized compounds have non-selectivity towards COX-1 or COX-2 enzymes. It is known that anti-inflammatory agents as indomethacin, sulindac, etodolac, ketorolac, and diclofenac, which bear arylacetic acid moiety, show nonselective COX enzyme inhibition²¹. Our finding supports this approach due to structural similarity between existing drugs and synthesized compounds that carry phenylacetic acid substructure. Due to lower IC₅₀ values of synthesized compounds than reference drugs ibuprofen and nimesulide, it can be concluded that thiazole ring have no influence on COX inhibition. Therefore, it can be suggested for further studies that new compounds, which include another ring system instead of thiazole may have more potent against COX enzymes. On the other hand, promising COX inhibition potencies of compounds 2b and 2d displayed the positive contribution of chloro substituent to pharmacological activity. Thus, incorporation of this substituent in the chemical structures of new compounds may cause beneficial contribution to COX inhibition^{13,14}.

Antimicrobial evaluation of the compounds revealed that synthesized compounds have potency against tested bacterial strains. It is thought that antibacterial activity of the compounds may be related to thiazole ring, which is one of the well-known ring system with antimicrobial capability^{11,13}. The compounds 2c and 2d have one more phenyl ring, than compound 2a and 2b on the chemical structure. This structural difference creates a lipophilicity distance between the compounds. Thus, it can be concluded that higher lipophilic character of the most active 2d was the main reason for increasing antibacterial activity. It can be suggested that synthesis of new compounds with similar lipophilic character to compound 2d may cause a development of new antibacterial agents.

In summary, evaluation of new 4-[4-(4-Substitutedphenyl)-5-substitutedthiazol-2-yl] phenylacetic acid derivatives as dual COX inhibitory-antibacterial agents resulted with hopeful findings. The compound 2d and displayed a good COX-1 and COX-2 inhibition along with significant antibacterial profile. In conclusion, findings of these

study will not only direct our research group to further studies, but also may have an researchers to synthesize more effective compounds bearing chemical structures similar to the compound 2d as dual COX inhibitory-antibacterial agents.

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