Synthesis and characterization of biologically active thiophene based amide motifs

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Abstract: Thiophene derivatives give medicinal chemists access to a combinatorial library and are essential precursors for the synthesis of heterocyclic molecules. S-heterocycles containing thiophene are thought to be useful medications for some conditions. Analog 1 and different anilines were combined with pyridine to create a range of thiophene derivatives (3a-f). Melting points, FT-IR, and ^{1}HNMR were used to characterize the structure and evaluate the cytotoxic activity of these compounds. Hemolysis and thrombolysis were analyzed in vitro to carry out the cytotoxic evaluation. Thiophene derivatives, S-heterocyclic compounds, hemolysis, thrombolysis, cytotoxic evaluation

Keywords: Thiophene derivatives, S-heterocyclic compounds, hemolysis, thrombolysis, cytotoxic evaluation

INTRODUCTION

Thiophene is an essential pharmacophore due to its diverse biological and pharmacological characteristics (da Cruz et al., 2021; Singh et al., 2020). Several investigations were conducted on the catalytic hydro desulfuration process and the biodegradation of thiophene (Omar et al., 2020). The coordination characteristics displayed are more akin to those of arenes when thiophene is used in place of thioether (Vallan et al., 2021). Amides, a carbonyl carbon is present in one of the three bonds (Vallan et al., 2021). As a result, according to (DeRuiter, 2005) amides are classified as "acylated amines" or derivatives of carboxylic acids, where the acid's -OH group is transformed to -NR_2, where R stands for hydrogen, alkyl, aryl, and so forth.
(Elder et al., 2008).
Biological features of nitrogen-bearing heterocycles, such as their potential for antibacterial (Nazeer et al., 2023; Shoaib et al.), antiviral, and anticancer effects (Noreen et al., 2022), make them highly significant (Biswas et al., 2024; Kaur et al., 2024). Thienopyrimidine pharmacological applications are widely recognized, particularly in relation to the antimicrobial properties of their corresponding thienopyrimidine compounds (Abdel-Megid et al., 2016; Lagardère et al., 2021; Tolba et al., 2017). Ticlopene, a well-known antibiotic penicillin, with a thiophene ring structure (Mabkhot et al., 2016). Tipepidine is used as an antitussive, and timepidium are anticholinergic and antispasmodic medications, respectively (Rudolph et al., 2008; Sasaki et al., 2014; Theriault et al., 2018) . Dorzolamide not only reduces glaucoma but also possesses a thiophene ring structure (Ghorai et al., 2020).
Considering these aspects and as a component of our continuous investigation into producing physiologically active heterocycles, we used a successful procedure to synthesis amide motifs based on thiophene in a sterile setting. . We then assessed the target molecules' pharmacological properties using thrombolysis and hemolysis.

MATERIALS AND METHOD

General Information

Only solvents and reagents of the highest caliber were employed in this synthesis sequence (Iftikhar et al., 2023; Khushnood et al.). The melting point (MP) of the target derivatives was determined using a Gallenkamp melting point apparatus (Snizhko et al., 2022). Bruker spectrometers were used to record NMR (Nuclear Magnetic Resonance) spectra at 400 MHz for 1H-NMR and 100 MHz for 13C-NMR (Kumar et al., 2021).
Parts per million, or ppm, were used to quantify chemical changes, whereas hertz (Hz) was used to assess coupling constant J values (Metz, 2016). FT-IR (Fourier transform-infra red) spectra were acquired using a Bruker Fourier Transform IR spectrometer (Pascale et al., 2022). To track the development of the reaction, silica gel plates were subjected to TLC (Thin Layer Chromatography). However, the UV (ultra violet) lamp (Booyens & Thantsha, 2014) caused spots to appear (Freeha Hafeez et al., 2022).

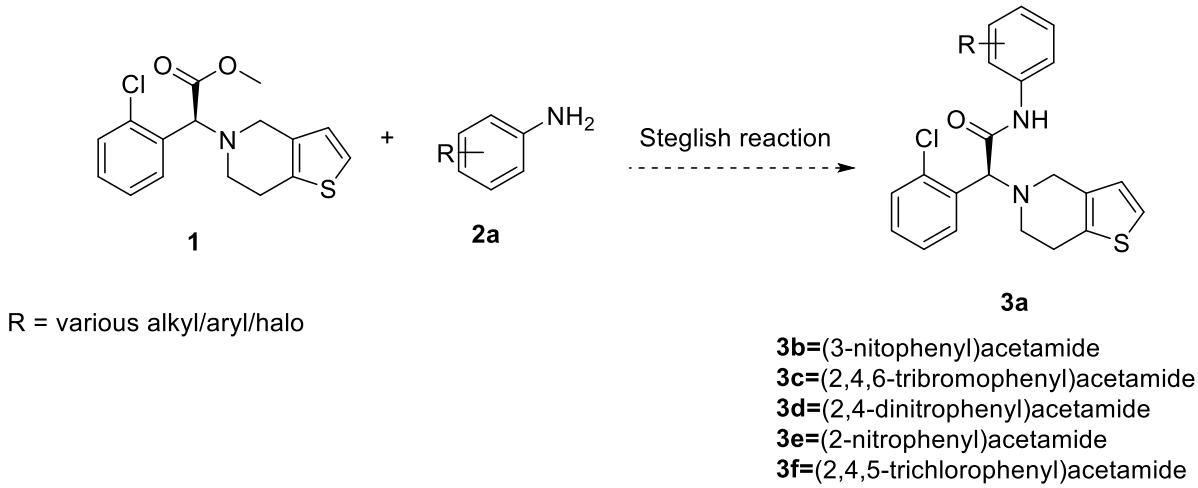

Figure 1: Synthesis of substituted aryl amides

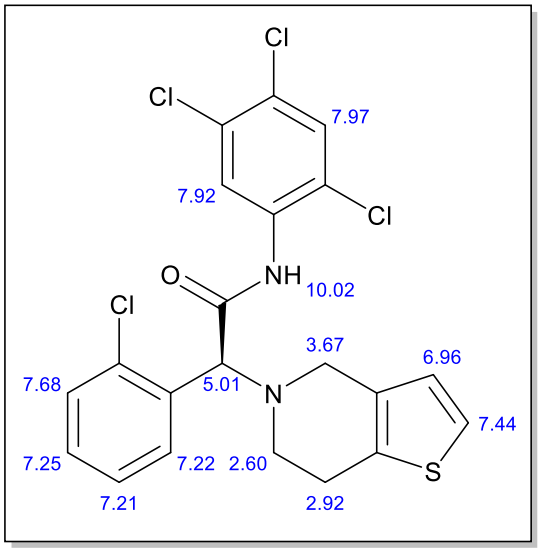

Figure 2: NMR interpretation of 3a

Synthesis of thiophene based amide motifs (3a)

(0.1g, 0.0003moles) Methyl(S)-2-(2-chlorophenyl)-2-(6,7-dihydrothio[3,2-c]pyridine-5(4H-yl)acetate and DCM (Dichloromethane) (10mL) with corresponding pyridine (0.12mL, 0.00075moles) at room temperature are simply mixed in this coupling process. After 48 hours at room temperature, the reaction medium produced outstanding yields (62–88%) of matching various anilines (2a). TLC was used to monitor the reaction, which had an ethyl acetate to n-hexane ratio of 7:3. After drying the organic phase. (Förtsch, 2018; Kanwal et al., 2022).

Biological Evaluation

Hemolysis

Red blood cells (RCBs) were separated by centrifuging a 50 mL blood sample. After being purified and treated with a DMSO sample solution (F Hafeez et al., 2022; Hafeez et al., 2021), the resultant supernatant was rinsed with PBS. After 60 minutes of incubation at 37°C, the tubes were centrifuged once more. Once the sample was diluted with phosphate buffer saline, the absorbance at 517 nm was determined. Three runs of the tests were conducted, and a formula was used to determine the percentage of RCB lysis (Antonio et al., 2015; Cao et al., 2021).

Thrombolysis

Experimented on thrombolysis using accepted techniques. A blood sample was given, and blood was placed into 500 µL Eppendorf tubes (Noor et al.; Siddique et al.). For one hour, the tubes were incubated at 37ºC to see the development of clots. After removing the serum, the tube holding the clot was weighed. To determine the proportion of cell lysis, a sample solution in DMSO was added to the clot in triplicate trials (Absar et al., 2013; Migliavacca et al., 2024).

Statistical Analysis

The statistical analysis was performed using Microsoft Excel 2010 and each experiment was conducted three times (AyeshaTariq et al.; Imran et al.). The data for cell viability are shown as the mean ± standard deviation (Sengupta et al., 2014).

RESULTS

By using dichloromethane (DCM) under solvent-free conditions, methyl(S)-2-(2-chlorophenyl)-2-(6,7-dihydrothio[3,2-c]pyridine-5(4H-yl)acetate with various substituted anilines (2a) produced excellent yields (62–88%) of thiophene-based amide motifs (3a–f) (Scheme 1) (Noureen et al.).

Hemolysis Analysis

Out of all the compounds tested, compound 172 caused the greatest amount of lysis of red blood cells, 5.7% more than the reference control. Moreover, it was the least cytotoxic. With rates of 1.45% and 3.80%, respectively, compounds 166 and 164 also significantly induced hemolysis. Compound 172 had the lowest level of cytotoxicity, at a 0.9% level.

The kind and position of the compounds' functional groups had a substantial impact on their hemolytic activity, indicating possible anticancer uses (Antonio et al., 2015; Cao et al., 2021).

Thrombolysis Analysis

We evaluated the potential for thrombolysis of each produced molecule. These compounds had relatively little thrombolytic action overall. Compounds 172 and 166 had more thrombolytic capability in comparison to the industry standard control, ABTS, whereas other derivatives shown intermediate capacity. Comparing Compound 170 to ABTS, Compound 170 had the highest lysis rate at 65.1%. The lowest lysis rate, however, was recorded by Compound 164, at 40.2%. In this paper, the impact of functional groups that positively induce thrombolysis is examined.
(Absar et al., 2013; Migliavacca et al., 2024).

DISCUSSION

Table 1: Spectral analysis of 3a-f

Compound	Melting Point	NMR Values
3b	170ºC	1H NMR spectrum shows peaks at δ 2.77–2.99 (4H, 2.85 (dd, J = 14.2, 10.0, 4.0 Hz), 2.86 (dd, J = 10.4, 7.0, 3.0 Hz), 2.92 (dd, J = 14.2, 4.1, 1.8 Hz)), 3.38–3.52 (2H, 3.45 (d, J = 16.6 Hz), 3.45 (d, J = 16.6 Hz)), 5.35 (1H, s), 7.01–7.17 (2H, 7.07 (d, J = 6.1 Hz), 7.10 (dd, J = 7.8, 7.5, 1.6 Hz)), 7.24–7.70 (7H, 7.30 (d, J = 8.2, 1.5 Hz), 7.45 (dd, J = 8.4, 8.2, 0.5 Hz), 7.55 (dd, J = 8.3, 1.6, 1.6 Hz), 7.63 (dd, J = 7.8, 1.3, 0.6 Hz)), 7.87 (1H, dd, J = 1.7, 1.5, 0.5 Hz)
3c	173ºC	1H NMR spectrum shows peaks at δ 2.77-2.99 (4H, 2.86 (dd, J = 14.2, 10.0, 4.0 Hz), 2.87 (dd, J = 10.3, 7.0, 3.0 Hz), 2.92 (dd, J = 14.2, 4.1, 1.8 Hz)), 3.38-3.53 (2H, 3.45 (d, J = 16.6 Hz), 3.46 (d, J = 16.6 Hz)), 5.35 (1H, s), 7.01-7.17 (2H, 7.07 (d, J = 6.1 Hz), 7.10 (dd, J = 7.8, 7.5, 1.5 Hz)), 7.20-7.36 (2H, 7.27 (dd, J = 8.3, 7.5, 1.3 Hz), 7.30 (d, J = 6.1 Hz)), 7.44 (2H, d, J = 1.5 Hz), 7.49-7.70 (2H, 7.55 (dd, J = 8.3, 1.5, 0.6 Hz), 7.64 (dd, J = 7.8, 1.3, 0.6 Hz))
3d	176ºC	1H NMR spectrum shows peaks at δ 2.72-3.00 (8H, 2.80 (dd, J = 10.4, 10.0, 4.1 Hz), 2.85 (dd, J = 14.2, 10.0, 4.0 Hz), 2.85 (dd, J = 14.2, 10.0, 4.0 Hz), 2.86 (dd, J = 10.4, 7.0, 3.0 Hz), 2.92 (dd, J = 14.2, 4.1, 1.8 Hz), 2.92 (dd, J = 14.2, 4.1, 1.8 Hz), 2.93 (dd, J = 10.4, 4.0, 1.8 Hz)), 3.38-3.52 (4H, 3.45 (d, J = 16.6 Hz), 3.45 (d, J = 16.6 Hz), 3.45 (d, J = 16.6 Hz), 3.45 (d, J = 16.6 Hz)), 5.25-5.35 (2H, 5.30 (s), 5.30 (s)), 7.01-7.17 (4H, 7.07 (d, J = 6.1 Hz), 7.07 (d, J = 6.1 Hz), 7.10 (dd, J = 7.8, 7.5, 1.5 Hz), 7.10 (dd, J = 7.8, 7.5, 1.5 Hz)), 7.24-7.39 (4H, 7.30 (d, J = 6.1 Hz), 7.30 (d, J = 6.1 Hz), 7.32 (dd, J = 8.3, 7.5, 1.3 Hz), 7.32 (dd, J = 8.3, 7.5, 1.3 Hz)), 7.49-7.70 (4H, 7.55 (dd, J = 8.3, 1.5, 0.6 Hz), 7.55 (dd, J = 8.3, 1.5, 0.6 Hz), 7.64 (dd, J = 7.8, 1.3, 0.6 Hz), 7.64 (dd, J = 7.8, 1.3, 0.6 Hz)), 7.97-8.09 (2H, 8.03 (dd, J = 7.8, 0.5 Hz), 8.03 (dd, J = 7.8, 0.5 Hz)), 8.32-8.44 (2H, 8.38 (dd, J = 7.8, 1.9 Hz), 8.38 (dd, J = 7.8, 1.9 Hz)), 8.72-8.83 (2H, 8.77 (dd, J = 1.9, 0.5 Hz), 8.77 (dd, J = 1.9, 0.5 Hz))

3e	174ºC	1H NMR spectrum shows peaks at δ 2.77-2.99 (4H, 2.85 (dd, J = 14.2, 10.0, 4.0 Hz), 2.86 (dd, J = 10.4, 7.0, 3.0 Hz), 2.92 (dd, J = 14.2, 4.1, 1.8 Hz)), 3.38-3.52 (2H, 3.45 (d, J = 16.6 Hz), 3.45 (d, J = 16.6 Hz)), 5.35 (1H, s), 7.01-7.17 (2H, 7.07 (d, J = 6.1 Hz), 7.10 (dd, J = 7.8, 7.5, 1.6 Hz)), 7.24-7.72 (7H, 7.30 (d, J = 6.1 Hz), 7.32 (dd, J = 8.3, 7.5, 1.3 Hz), 7.37 (dd, J = 8.0, 7.5, 1.5 Hz), 7.47 (dd, J = 8.3, 1.5, 0.5 Hz), 7.55 (dd, J = 8.3, 1.6, 0.6 Hz), 7.64 (dd, J = 7.8, 1.3, 0.6 Hz), 7.65 (dd, J = 8.3, 7.5, 1.8 Hz)), 8.08 (1H, dd, J = 8.0, 1.8, 0.5 Hz)
3f	178ºC	1H NMR spectrum shows peaks at δ 2.72-3.00 (8H), including peaks at 2.80 (dd, J = 10.4, 10.0, 4.1 Hz), 2.86 (dd, J = 14.2, 10.0, 4.0 Hz), 2.86 (dd, J = 14.2, 10.0, 4.0 Hz), 2.86 (dd, J = 10.4, 7.0, 3.0 Hz), 2.92 (dd, J = 14.2, 4.1, 1.8 Hz), 2.92 (dd, J = 14.2, 4.1, 1.8 Hz), and 2.93 (dd, J = 10.4, 4.0, 1.8 Hz). There are also signals at δ 3.38-3.53 (4H), with peaks at 3.45 (d, J = 16.6 Hz), 3.45 (d, J = 16.6 Hz), 3.46 (d, J = 16.6 Hz), and 3.46 (d, J = 16.6 Hz). Additionally, there are signals at δ 5.30-5.40 (2H), with peaks at 5.35 (s) and 5.35 (s). The spectrum also shows signals at δ 7.01-7.17 (4H), with peaks at 7.07 (d, J = 6.1 Hz), 7.07 (d, J = 6.1 Hz), 7.10 (dd, J = 7.8, 7.5, 1.5 Hz), and 7.10 (dd, J = 7.8, 7.5, 1.5 Hz). Furthermore, there are signals at δ 7.20-7.36 (6H), with peaks at 7.27 (dd, J = 8.3, 7.5, 1.3 Hz), 7.27 (dd, J = 8.3, 7.5, 1.3 Hz), 7.30 (d, J = 6.1 Hz), 7.30 (d, J = 6.1 Hz), 7.35 (d, J = 7.8, 1.3 Hz), 7.35 (d, J = 7.8, 1.3 Hz)

Table 2: Target molecule hemolysis and thrombolysis potential mean ± standard deviation (3a-f)

Compounds	Hemolysis (% ± SD) a*	Thrombolysis (% ± SD) a*
3b	2.05 ± 0.121	47.2 ± 0.081
3c	1.45 ± 0.004	65.1 ± 0.081
3d	3.80 ± 0.005	40.2 ± 0.081
3e	5.7 ± 0.041	54.2 ± 0.081
3f	0.9 ± 0.009	30.5 ± 0.081
ABTS(Positive control)	95.9	86

Amidomotifs (3a–f), a synthesized thiophene-based moiety, were produced in excellent yields (62–88%) by starting DCM as a versatile medium under solvent-free conditions, where different substituted anilines 20 were applied to methyl(S)-2-(2-chlorophenyl)-2-((6,7-dihydrothio[3, 2-c]pyridin-5(4H) - yl) acetate. All five proteins' structural integrity was confirmed by NMR data.

The hemolysis tests showed that compound 3e induced the highest levels of hemolysis (5.7%), whereas compounds 3d (3.80%) and 3b (2.05%) caused moderate, intermediate effects; meanwhile, there was a low level for 0.9% observed with pH =6-introduced succinate analogs compared to PBS control group as it seems initial assessment of functional groups on their impact is highly variable regarding facial presentation downstream capabilities by those moiety served or buried influencing in potentiation discriminatory sense. These differences are important for the future design of new and safer anticancer agents.

Compound 3c was the most effective among our series of thrombolysis evaluations, it exhibited 65.1% inhibition and even higher than standard control ABTS (86%) indicating excellent potential as a therapeutic agent for thrombolysis. Therefore, compound 3d possessed minimum thrombolytic activity (40.2%). Structural modification Studies demonstrated the increased thrombolytic efficacy of acyl hydrazines with functional groups having positive inductive effects, underscored by their role.

Correlation is established between the ability of compounds to cleave blood clot and destroy red cell membrane for hemolysis, formation of yellow rings at end point with substitution on thiophene ring that influence both inhibitory actions. Compounds 3b and 3c showed moderate hemolytic (HL) activity but appreciated thrombolytic activities in comparison to reference drug, making them promising leads. Among stable hybrid peptide/fatty hydroxamic acid complexes, compound 3f (minimal hemolysis in combination with moderate thrombolytic activity) displayed a good antithrombotic profile despite the cytotoxicity detected.results and discussion

CONCLUSION

Functionalization of the synthesized thiophene-based

amides motifs led to substantial variations in hemolytic and thrombolytic activities. The highest hemolysis rate was observed with compound 3e, which suggests the potential for cytotoxicity; in contrast to those findings, minimal hemolysis is shown by compounds like 3f that put forward lower toxicity. Compound 3c exhibited the maximum lysis rate in thrombolytic activity, which was superior to other compounds showing significant therapeutic prospects. The importance of functional groups in the determination and control of biological activity was greatly highlighted by these results. The study identifies compound 3c as a best thrombolytic agent whereas the compounds exhibited minimal hemolysis, however among them, only compound 3f depicts least degree of haemodynamic activity and demonstrated these two candidates are promising molecules for future drug design in therapeutic purposes. Structure-activity relationship Studies have drawn attention to the importance of specific structural alterations in thiophene-based amide scaffolds with regard to protective abilities and imply a potential strategy for optimization for therapeutic applications.

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