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Facile green approach towards the synthesis of some phenyl piperazine based dithiocarbamates as potent hemolytic and thrombolytic agents

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Abstract: The facile and efficient protocol for the synthesis of *N*-phenyl piperazine based di-thio-carbamates has been reported under neat conditions. A library of novel piperazine based di-thio-carbamates (3a-h) in excellent yields has been prepared. Solvent free, catalyst free and easy work up conditions make this protocol an attractive synthetic protocol to achieve novel biologically active di-thio-carbamates. The synthesized molecules have been characterized by FT-IR, ^{1}HNMR and ^{13}CNMR spectroscopic techniques. The pharmacological aspects of these derivatives have been evaluated *via* hemolysis and thrombolysis. All the target molecules (3a-h) exhibit mild to medium potential as hemolytic and thrombolytic agents. Among the synthesized derivatives, compound 3c showed least cytotoxicity and better thrombolytic potential.

Keywords: *N*-Phenyl piperazine, hemolysis, thrombolysis, neat conditions, di-thio-carbamates.

INTRODUCTION

Synthetic strategies involving organic and haloalkane solvents, cause a serious risk to environment (Wegner *et al.,* 2021). Therefore, it is the need of time to design greener and solvent free synthetic pathways (Saaed *et al.,* 2020). In general, the use of catalysts in many cases may also pose a threat to the environment due to waste disposal associated with the use of organic solvents (Tanaka *et al.,* 2000). To minimize these hazards, neat reaction conditions often provide a sustainable alternate synthetic pathway towards the synthesis of a variety of organic molecules (Sridhar *et al.,* 2020; Srivastava *et al.,* 2020) which are solvent free (Back *et al.,* 2020; Zhang *et al.,* 2020) and can also be catalyst free reaction conditions. Also, mild conditions and easy workups are generally associated with such methodologies (Lillo *et al.,* 2018; Mahato *et al.,* 2018; Li *et al.,* 2020).

Nitrogen bearing heterocycles, are of considerable significance because of their promising biological properties such as antibacterial, antiviral, and anticancer potential (Singh *et al.,* 2020; Gupta *et al.,* 2020; Akhtar *et al.,* 2021). Among *N*-heterocycles, piperazine scaffold have emerged as pharmacological important moiety (Zahoor *et al.,* 2017; Akhtar *et al.,* 2016; Hafeez *et al.,*2018) with broad spectrum biological potential *viz.* anticancer (Akhtar *et al.,* 2019; Hafeez *et al.,* 2021). Some of the piperazine based drugs have been displayed in fig. 1.

Furthermore, di-thio-carbamate framework plays a significant role as intermediate in the synthesis of various heterocycles (Foumeshi *et al.,* 2020). Di-thio-carbamate based organic molecules possess wide range of pharmacological activities such as anticancer, antibacterial and antiviral effect (Asar *et al.,* 2020). Di-thio-carbamate motif is member of several anticancer drugs (Arsakhant *et al.,* 2020).

Keeping in view these considerations and in continuation of our research towards the synthesis of novel biologically active heterocycles, herein, we utilized an efficient protocol for the synthesis of piperazine based di-thio-carbamates in neat conditions and evaluated the pharmacological aspects of these target molecules *via* hemolysis and thrombolysis.

MATERIALS AND METHODS

General information

All the reagents and solvents employed in this synthetic sequence were of analytical grade. MP (Melting point) of the target derivatives was estimated on Gallenkamp melting point apparatus. NMR (Nuclear Magnetic Resonance) spectra was recorded on Bruker spectrometer (400 MHz, ^{1}H-NMR) and (100 MHz, ^{13}C-NMR). Chemical shifts were expressed in ppm (parts per million), while coupling constant J values were given in Hertz (Hz). FT-IR (Fourier transform-infra red) spectra were obtained using Bruker Fourier Transform IR spectrometer. TLC (Thin Layer Chromatography) was carried out on silica gel plates, to monitor the reaction progress. However, spots were developed under UV (Ultra violet) lamp.

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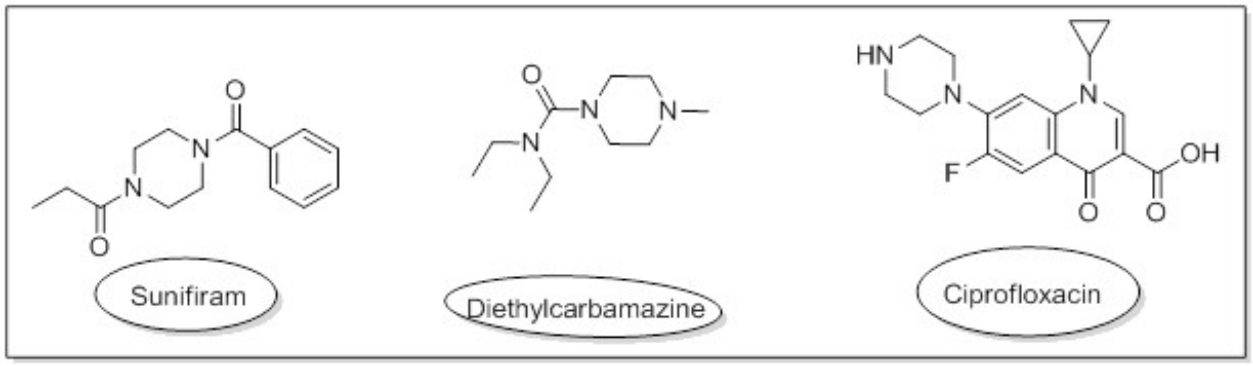
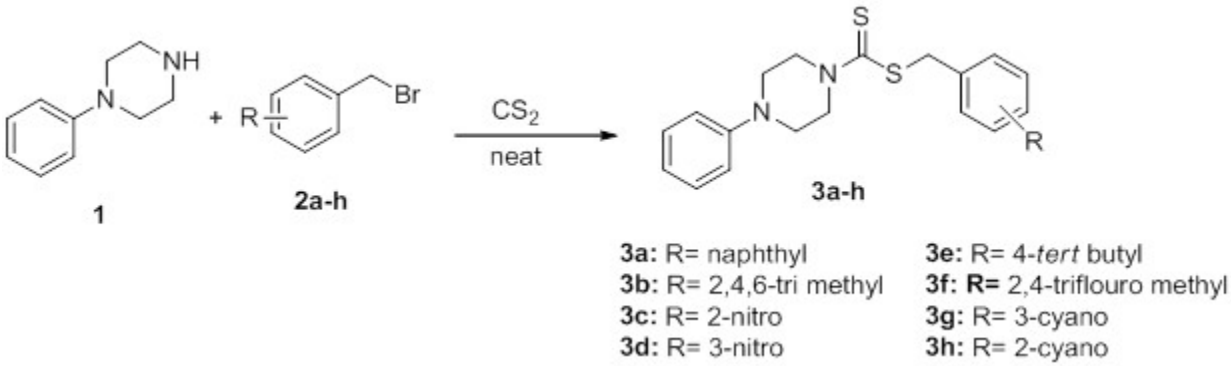


Fig. 1: Structures of piperazine based drugs



Scheme 1: Synthesis of target molecules (3a-h)

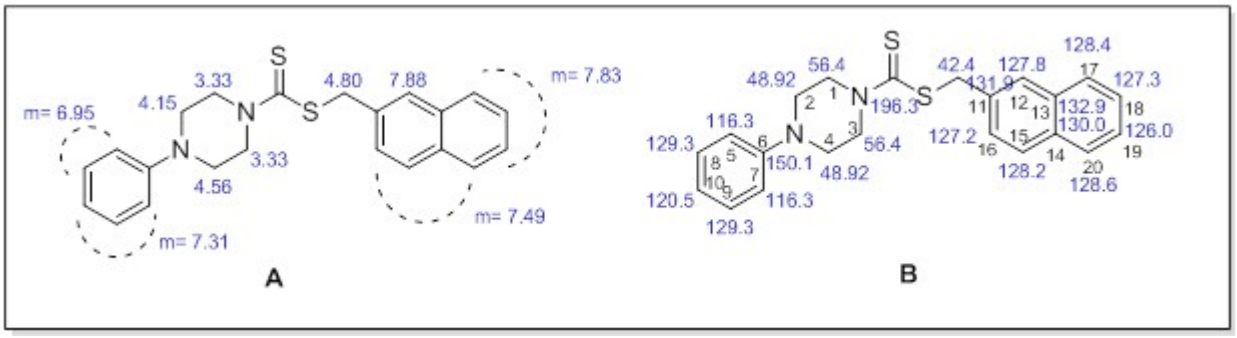


Fig. 2: NMR Interpretation of 3a

Common Experimental Procedure for the synthesis of piperazine-based di-thio-carbamates (3a-h)

This coupling methodology involves the simple mixing of *N*-phenyl piperazine (0.93 mmol) and CS_2 (Carbon disulfide) (0.93 mmol) with corresponding benzyl bromide (0.46 mmol) at room temperature. The reaction medium was allowed, to stand at room temperature for 12 hours leading to corresponding phenyl piperazine based dithiocarbamates (3a-h) in excellent yields (80-95%). Progress of reaction was monitored by TLC. On completion of reaction, extraction of organic phase was carried out using ethyl acetate (3x30mL) and dried the organic phase over sodium sulfate and crude product was afforded after evaporation. Pure product was obtained on crystallization with ethanol (Azizi *et al.,* 2006).

Biological Evaluation

Hemolysis

In vitro hemolytic studies of the synthesized derivatives was carried out *via* literature methodology (Shahzadi *et al*., 2021). Fresh blood sample (7 mL) from healthy human donor was collected in EDTA (Ethylenediaminetetraacetic acid) tube. Blood was transferred to micro centrifuge tubes and centrifuged the red blood cells (RBCs) for 5 minutes at 1000 rpm. Then supernatant was discarded, and RBCs pellet was washed with phosphate buffer saline (PBS) three times. The RBCs pellet was collected after washing and 20µL sample solution in DMSO (Dimethyl sulfoxide) was added. The tubes were incubated at 37 ^{o}C for 60 minutes. After removing tubes from incubator, again centrifuged them, at 13000 rpm for 5 minutes. Collected the supernatant and diluted with chilled PBS solution. Record

the absorbance at 517 nm. ABTS (2-2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was used as positive control while DMSO (Dimethyl sulfoxide) was employed as negative control in this protocol. Experiments were conducted in triplicates and % RBC lysis was calculated by using this formula (Shahzadi *et al.,* 2020).

$$\% \text{ age hemolysis} = \frac{\text{Absorbance of sample} - \text{Absorbance of negative control}}{\text{Absorbance of positive control}} \times 100$$

Thrombolysis

The thrombolytic assay was performed *via* literature methodology (Batool *et al.,* 2018) utilizing in vitro technique. The blood sample (3mL) was collected from healthy human donor and 500 µL was transferred to pre-weighed clean eppendorf tubes. Again, weighed these tubes with blood and for clot formation, incubate these tubes for 1 hour at 37 ^{o}C. Then the serum was discarded and tube with clot was weighed. 40 µL of sample solution in DMSO was added to the clot and again incubate these tubes for 3 hours at 37 ^{o}C and observed the lysis results. ABTS was used as negative control, while DMSO was used as negative control in this assay. The experiments were performed in triplicate and % lysis was calculated using the formula below (Shahzadi *et al.,* 2020):

$$\% \text{ age clot lysis} = \frac{\text{Initial clot weight} - \text{Final clot weight}}{\text{Initial clot weight}} \times 100$$

STATISTICAL ANALYSIS

All the experiments were conducted in triplicates and expressed as mean ± SD. Microsoft Excel 2010 was used for statistical analysis. One way ANOVA was performed for comparing results and $p \leq 0.05$ was considered significant.

RESULTS

Synthesis of Phenyl piperazine based dithiocarbamates

Coupling of *N*-phenyl piperazine 1 with various substituted benzyl halides (2a-h) using carbon disulfide (CS_2) in solvent free conditions, was carried out to afford phenyl piperazine based dithiocarbamates (3a-h) in excellent yields (80-95%) (Scheme 1) (Azizi *et al.,* 2006).

Hemolysis

Hemolysis of all the target molecules (3a-h) was carried out using *in vitro* technique. When compared with positive control ABTS, compound 3c exhibited least cytotoxicity (0.1%), while 3b (7.05%) showed highest toxicity. The least cytotoxic effect of 3c is due to the presence of nitro group which have electron withdrawing effect, in respect of resonance and inductive effect both. Other derivatives such as 3d (0.35%), 3g (0.72%) and 3h (0.66%) displayed moderate hemolysis when compared with ABTS (positive control). However, highest cytotoxicity is exhibited by 3a (6.14%), 3b (7.05%), 3e (2.6%) and 3f (1.68%), this effect may be due to presence of bulky groups on alkyl ring (table 2).

Thrombolysis

All the synthesized derivatives (3a-h) were investigated for their anticoagulant effect utilizing *in vitro* technique. Among these molecules, compound 3b, 3c and 3h displayed better thrombolytic potential, while other compounds 3a, 3d-e, and 3g exhibited mild to average % anticoagulant effect as compared to ABTS (positive control). Piperazine based derivative 3c exhibited highest % lysis (65.4%) when compared with control (ABTS), the effect is associated with the presence of electron withdrawing nitro group on the ring. However, 3b and 3g displayed average potential values viz. 62.5% and 60.4% respectively. The data from table 2 also revealed that 3f showed least % lysis which accounts for the presence of bulky trifluoro groups at *ortho-para* position of phenyl ring. Furthermore, it is also recommended that presence of electron withdrawing group on phenyl ring enhance the thrombolytic effect.

DISCUSSION

Spectral characterization of derivative 3a was conducted *via* FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. In the FT-IR spectrum, characteristic absorption band of (Carbon double bond sulfur) appears at 1224 cm^{-1}, while C=C exhibited peak at 1524 cm^{-1}. Proton NMR spectrum of 3a gave triplet at 3.33 ppm for 4 hydrogens of piperazine, however 2 protons of piperazine appeared as broad singlet at 4.15 ppm, while 2 hydrogens of piperazine showed at chemical shift of 4.56 ppm. Protons of linker (methylene) appeared at 4.80 ppm. Multiplets appeared at 6.95 and 7.31 ppm for 3 and 2 hydrogens of phenyl ring respectively. Furthermore, protons of naphthyl ring gave chemical shifts in the following pattern: singlet at 7.88ppm, while multiplets appeared at 7.83ppm and 7.49ppm. Carbon NMR spectrum of 3a gave significant C=S peak at 196.3 ppm. C1-C3 and C2-C4 peaks, which appeared at 56.4 ppm and 48.92 ppm represents carbons associated with piperazine. Characteristic peak at 42.4 ppm exhibited for S-CH_2. However, peaks at 116.3, 120.5, 129.3 and 150.1 ppm were linked with carbons of aryl ring while naphthyl ring carbons appeared in the region 132.9, 131.9, 130.0, 128.6, 128.4, 128.2, 127.3, 127.2 and 126.0 ppm. All the other target molecules were elucidated in the same protocol.

All the synthesized derivatives were investigated for their pharmacological profile. The results for the cytotoxic potential i.e. hemolysis and thrombolysis assay are summarized in table 2 which revealed that presence of

Table 1: Spectral studies of piperazine based dithiocarbamates (3a-h) in green conditions

Compound	MP (^oC)	Yield (%)	FT-IR (cm^{-1})ν_{max} / ^{1}HNMR (500 MHz, $CDCl_3$)/ ^{13}C NMR (100 MHz, $CDCl_3$)/ MS (EI) (m/z)
3a	120	91	1524 (C=C), 1450 (CH_2), 1224 (C=S)/7.88 (s, 1H, ph-H), 7.83 (m, 3H, Ph-H), 7.49 (m, 3H, Ph-H), 7.31 (m, 3H, Ph-H), 6.95 (m, 2H, Ph-H), 4.80 (s, 2H, S-CH_2), 4.56 (bs, 2H, piperazine), 4.15 (bs, 2H, piperazine) 3.33 (t, 4H, J= 4Hz, piperazine)/196.3 (C=S), 150.1, 132.9, 131.9, 130.0, 129.3, 128.6, 128.4, 128.2, 127.3, 127.2, 126.0, 120.5, 116.3 (Ph-C), 48.92 and 56.4 (N-CH_2 piperazine), 42.44 (S-CH_2)/379.1302 [M+1]
3b	134	86	1522 (C=C), 1450 (CH_2), 1234 (C=S)/7.3 (m, 2H, ph-H), 6.95 (m, 5H, Ph-H), 4.51 (s, 2H, S-CH_2), 3.69 (t, 4H, J=4Hz, piperazine), 3.32 (bs, 4H, piperazine), 2.39 (s, 6H, CH_3), 2.29 (s, 3H, CH_3)/196.3 (C=S), 150.1, 137.2, 135.1, 129.3, 127.8, 120.5, 116.3 (Ph-C), 48.9 and 50.1 (N-CH_2 piperazine), 42.44 (S-CH_2), 20.9 and 18.3 (CH_3)/371.1615 [M+1]
3c	164	95	1521 (C=C), 1453 (CH_2), 1220 (C=S)/8.3 (d, 1H, J=4Hz,Ph-H), 8.1 (dd, 1H, J=8Hz, ph-H), 7.7 (d, 1H, J= 4Hz, Ph-H), 7.5 (dd, 1H, J= 8Hz,Ph-H), 7.31 (dd, 2H, J= 8Hz, Ph-H), 6.95 (m, 3H, Ph-H), 4.74 (s, 2H, S-CH_2), 4.53 (bs, 2H, piperazine), 4.13 (bs, 2H, piperazine), 3.35 (t, 4H, J= 4Hz piperazine-H)/196.3 (C=S), 150.1, 149.8, 135.1, 133.0, 132.1, 129.3, 128.1, 125.1, 120.5, 116.3 (Ph-C), 48.9 and 50.1 (N-CH_2 piperazine), 42.44 (S-CH_2)/374.0996 [M+1]
3d	162	90	1528 (C=C), 1455 (CH_2), 1220 (C=S)/8.3 (s, 1H, Ph-H), 8.1 (d, 1H, J=8Hz, Ph-H), 7.9 (m, 2H, ph-H), 7.3 (dd, 2H, J=8Hz, Ph-H), 6.95 (m, 3H, Ph-H), 4.74 (s, 2H, S-CH_2), 4.53 (bs, 2H, piperazine), 4.13 (bs, 2H, piperazine), 3.35 (t, 4H, J= 4Hz, piperazine)/196.3 (C=S), 149.95, 148.5, 133.9, 133.7, 132.97, 129.91, 128.55, 125.85, 120.86, 117.19 (Ph-C), 48.9 and 50.04 (N-CH_2 piperazine), 38.67 (S-CH_2)/374.0996 [M+1]
3e	108	80	1528 (C=C), 1450 (CH_2), 1240 (C=S)/7.5 (d, 2H, J= 8Hz, Ph-H), 7.3 (dd, 2H, J= 8Hz, ph-H), 7.2 (d, 2H, J= 8Hz, Ph-H), 6.95 (m, 3H, Ph-H), 4.74 (s, 2H, S-CH_2), 4.53 (bs, 2H, piperazine), 4.13 (bs, 2H, piperazine), 3.35 (t, 4H, J= 4Hz, piperazine), 1.37 (s, 9H, CH_3)/196.3 (C=S), 150.63, 139.93, 132.93, 129.54, 125.55, 120.87, 116.88 (Ph-C), 48.97 and 50.02 (N-CH_2 piperazine), 42.44 (S-CH_2), 34.96 ($C(CH_3)_3$), 31.59 (CH_3)/385.1771 [M+1]
3f	105	81	1520 (C=C), 1455 (CH_2), 1230 (C=S)/8.0 (s, 1H, Ph-H), 7.7 (s, 2H, ph-H), 7.3 (dd, 2H, J= 8Hz, Ph-H), 6.95 (m, 3H, Ph-H), 4.74 (s, 2H, S-CH_2), 4.53 (bs, 2H, piperazine), 4.13 (bs, 2H, piperazine), 3.35 (t, 4H, J= 4Hz, piperazine-H)/196.3 (C=S), 150.1, 141.2, 132.1, 129.3, 129.1, 121.7, 120.5, 116.3 (Ph-C), 125.1 (CF_3) 48.9 and 50.1 (N-CH_2 piperazine), 42.44 (S-CH_2)/465.0893 [M+1]
3g	150	80	1528 (C=C), 1455 (CH_2), 1240 (C=S)/8.0 (s, 1H, Ph-H), 7.9 (m, 2H, Ph-H), 7.3 (dd, 2H, J= 8Hz, Ph-H), 7.1 (dd, 1H, J= 8Hz, Ph-H) 6.95 (m, 3H, Ph-H), 4.74 (s, 2H, S-CH_2), 4.53 (bs, 2H, piperazine), 4.13 (bs, 2H, piperazine), 3.35 (t, 4H, J= 4Hz, piperazine)/196.3 (C=S), 150.1, 140.9, 132.5, 132.1, 129.3, 129.1, 120.5, 116.3 (Ph-C), 119.1 (C triple bond N), 113.2 (Ph-C triple bond N), 48.9 and 50.1 (N-CH_2 piperazine), 42.44 (S-CH_2)/354.1098 [M+1]
3h	128	85	1518 (C=C), 1452 (CH_2), 1239 (C=S)/7.7 (dd, 1H, J= 8Hz, Ph-H), 7.6 (d, 1H, J=8Hz, Ph-H), 7.56 (dd, 1H, J= 8Hz, Ph-H), 7.39 (dd, 1H, J= 8Hz, Ph-H), 7.3 (dd, 2H, J= 8Hz, Ph-H), 6.95 (m, 3H, Ph-H), 4.74 (s, 2H, S-CH_2), 4.53 (bs, 2H, piperazine), 4.13 (bs, 2H, piperazine), 3.35 (t, 4H, J= 4Hz, piperazine)/196.3 (C=S), 150.1, 142.5, 133.9, 132.2, 129.3, 128.2, 127.5, 120.5, 116.3 (Ph-C), 119.1 (C triple bond N), 113.2 (Ph-C triple bond N), 48.9 and 50.1 (N-CH_2 piperazine), 42.44 (S-CH_2)/354.1098 [M+1]

Table 2: Mean ± S.D values of hemolysis and thrombolysis potetnial of target molecules (3a-h)

Compound	Hemolysis (% ± SD) [a*]	Thrombolysis (% ± SD) [a*]
3a	6.14 ± 0.03	57.2 ± 0.26
3b	7.05 ± 0.04	62.5 ± 0.1
3c	0.12 ± 0.02	65.4 ± 0.41
3d	0.35 ± 0.02	59.3 ± 0.26
3e	2.6 ± 0.05	55.2 ± 0.25
3f	1.68 ± 0.01	49.3 ± 0.2
3g	0.72 ± 0.03	50.0 ± 0.05
3h	0.66 ± 0.02	60.4 ± 0.2
ABTS (positive control)	95.9	86

[a]Experiments were performed in triplicates and expressed as mean ± SD
[*] $p \leq 0.05$, was considered significant

electron withdrawing nitro group enhance the cytotoxic potential of these derivatives. Among all the derivatives the % hemolytic potential of compound 3c was 0.12% which significantly lower than other synthesized derivatives while 3c also exhibited better thrombolytic potential with 65.4 % value which is highest than other synthesized derivatives.

CONCLUSION

Owing to the pharmacological significance of piperazine and di-thio-carbamate moiety, we have utilized an efficient one pot protocol for the coupling reaction of *N*-phenyl piperazine and di-thio-carbamate in neat conditions. This synthetic strategy due to its cost effectiveness and green conditions provides a facile route for the synthesis of piperazine based derivatives in excellent yields (80-95%). The target molecules (3a-h) were evaluated for their biochemical profile *via* hemolysis and thrombolysis. Among all the synthesized molecules, 3c exhibited least cytotoxicity and also, appeared as effective thrombolytic agent and represents effective lead derivative for future studies.

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