EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 7
569-574
Year: 2018

SYNTHESIS AND EVALUTION OF MUTUAL PRODRUGS FROM CLOPIDOGREL ANALOGUES AND SALICYLIC ACID

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Article Received on 09/05/2018 **Article Revised on 29/05/2018** **Article Accepted on 19/06/2018**

ABSTRACT
A series of 4,5,6,7- tetrahydrotheino[3,2-c]pyridine derivatives having structure analogs to clopidogrel was synthesized and prepared their mutual prodrug with salicylic acid and evaluated for anti-platelet and antithrombotic activity. The confirmation of structure was done by IR, NMR, and Mass fragmentation. All synthesized mutual prodrug were subjected to investigation for their anti-platelet and antithrombotic activity using clopidrogrel and aspirin as the standard drugs alone or in combination, synthesized mutual prodrug of clopidogrel analogues and salicylic acid shows better activity as compared to standard drugs.

KEYWORDS: Clopidogrel, salicylic acid, anti-platelet activity and antithrombotic activity.

INTRODUCTION

Several antiplatelet and antithrombotic agent are available in the market amongst them clopidogrel and aspirin are orally active inhibitor of platelet aggregation and antithrombotic agent. Clopidogrel is an adenosine diphosphate (ADP) receptor antagonist used for the reduction of myocardial infarction, ischemic heart disease, and vascular death.[2] Where as, aspirin inhibits cyclooxygenase activity and affect the production of thromboxane TXA_2. Several antiplatelet agents with different mechanisms of action are currently available for secondary prevention of ischemic stroke.[3,6] When used as a single agent, the efficacy of antiplatelet therapy is modest. Aspirin is the best-studied and most widely used antiplatelet agent for stroke prevention; however, it provides only an approximately 15% relative risk reduction for secondary prevention of stroke or other major vascular events. Combining two antiplatelet agents with different mechanisms of action was demonstrated to provide a substantial increase in efficacy.[6,12] In recent years, there have been an increasing an interest in the design and development of mutual prodrugs, which involves combining of two different pharmacophores with similar pharmacological activities which may give synergistic action. Therefore an attempt has been made to adjoin their different derivatives through an ester linkage to form a series of mutual prodrugs which were further screened physiochemical and pharmacologically. This may improve acceptability of the compound by patient in the final stage along with their additive effect against platelet aggregation & thrombus formation. Clopidogrel contains 4,5,6,7-tetrahydrothieno[3,2-c]pyridine as a basic ring. The structure of proposed derivatives was given in Fig: 1.

Compound	R_1	R_2
I	2-Cl	H
II	2-Cl	3-Br
III	2-Cl, 4-Cl	H
IV	2-Cl, 4-Cl	3-Br,5-Br

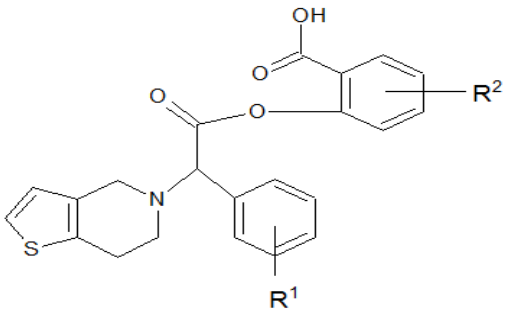


Fig. 1: Structure of proposed derivative.

MATERIALS AND METHODS

The chemicals used in the present work were AR grade and LR grade, purchased from Loba, Merck and Fisher scientific fine chemicals.

Mutual prodrugs of clopidogrel analogues and aspirin were synthesized as outline in Scheme1. The commercially available phenyl acetonitrile (1) serve as a convenient starting material in the synthesis on bromination gives 2-bromo-2-phenyl acetonitrile (2) which react with 4,5,6,7 tetrahydrothieno (3,2-c)pyridine

and yielded 2-phenyl 2-[4,5,6,7-tetrahydrothieno (3,2-c)pyridine]acetonitrile (4) which convert into corresponding acid (5) and by reaction with thionyl chloride it gives corresponding acid chloride (6). The compounds (6) react with salicylic acid and their derivatives in the presence of dry pyridine to yielded mutual prodrug of clopidogrel analogues (7).

The structure of the compounds were confirmed by Infrared spectroscopy, Nuclear Magnetic Resonance spectroscopy and Mass spectroscopy. The melting point of the synthesized compounds were determined in open capillary using LABHOSP melting point apparatus and recorded in ^{o}C without correction. Thin layer chromatography was performed on recoated silica gel plates (60 GF254 Merck) with suitable solvent system. The R_f values were recorded accordingly. The IR spectra were recorded using SHIMADZU-FTIR 8400 spectrophotometer using potassium bromide pellet technique and sodium chloride cells for liquid samples. H^1NMR spectra were taken using Bruker ACF-300 MHz spectrometer using tetramethyl silane (TMS) as an internal standard. H^1NMR spectra were recorded with $CDCl_3$ as a solvent and the chemical shift data were expressed as δ values relative to TMS and Mass spectra were recorded using SHIMADZU GCMS-2010 mass spectrometer. All animals obtained from the animal house of J. L. Chaturvedi College of Pharmacy, Nagpur, India.

Synthesis

Step-1: General procedure for the preparation of 2-Bromo-2phenyl acetonitrile (2 I-II)

Bromine (8.8 g, 0.055 mol) was added over a period of 3 hrs to 2-phenyl acetonitrile (7.57 g,0.05 mol) with stirring at 105-110^{0}C and was reacted for another 3 hrs. Then butyl acetate (12.5ml) and sodium bisulfate (0.57 g,) were added at 30^{0}C. After 15 min stirring, the mixture was filtered, washed with water, recrystallized with isopropyl alcohol and dried over anhydrous sodium sulfate to give 2-bromo-2phenyl acetonitrile, yield 6 g, m.p 111^{0}C.

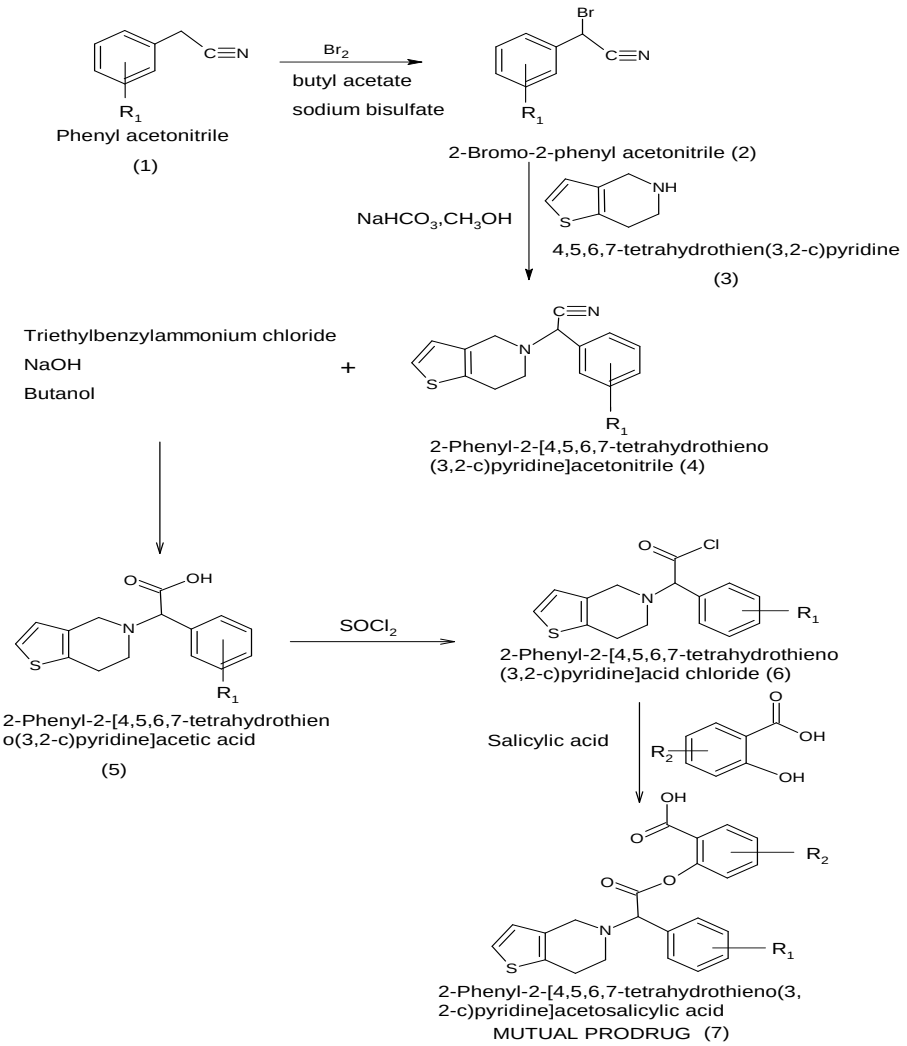


Fig. 2: Scheme-1 Synthetic routes of compounds (1-7).

Step-2: General procedure for the preparation of 2-phenyl-2-[4,5,6,7-tetrahydrothieno(3,2-c)pyridine] acetonitrile (4 I-II)

Compound 2-Bromo-2-phenyl acetonitrile (4.93g0.021mol), 4,5,6,7-tetrahydrothieno (3,2-c)pyridine (3.51 g, 0.2 mol) Sodium hydrogen carbonate (4.2 g,0.05 mol) and methanol (15 ml) were combined and refluxed for 3 hrs. Then the mixture was stirred at 5 ^{0}C for more than 30 min, filtered washed with water and cold methanol, recrystallized with isopropyl alcohol and dried to give 2-phenyl 2-[4,5,6,7-tetrahydrothieno (3,2-c)pyridine]acetonitrile.

Step-3: **General procedure for the preparation of 2-phenyl-2-[4,5,6,7-tetrahydrothieno(3,2-c)pyridine] acetic acid (5 I-II)**

The mixture of 2-phenyl 2-[4,5,6,7-tetrahydrothieno (3,2-c)pyridine]acetonitrile (3 g, 0.011 mol) TEBA (0.05 g), sodium hydroxide (18 g, 40-50%) and butanol (5ml) was refluxed for 12 hrs. After cooling to room temperature, the solution was neutralized with HCl (35-37%) to pH 8 and then acidified with acetic acid to pH 4-5. The suspension was filtered washed and recrystallized with isopropyl alcohol then dried to give 2-phenyl 2-[4,5,6,7-tetrahydrothieno (3,2-c) pyridine]acetic acid.

Step-4: General procedure for the preparation of 2-phenyl 2-[4,5,6,7-tetrahydrothieno (3,2-c)pyridine] acid chloride (6 I-II)

In 250 ml round bottom flask a mixture of 2-phenyl 2-[4,5,6,7-tetrahydrothieno (3,2-c) pyridine]acid chloride(1.0 g) and redistilled thionyl chloride (5 ml) was taken then condenser having $CaCl_2$ guard tube was fitted as a gas absorption trap connected and start gentle heating on water bath for 40-50 min. Then the solution allows cooled, filtered and crude product was recrystallized using isopropyl alcohol.

Step-5: General procedure for the preparation of 2-phenyl 2-[4,5,6,7-tetrahydrothieno(3,2-c)pyridine] acetosalicylic acid (7 I-IV)

In 250 ml round bottom flask 0.4 g mixture of 2-(2-chlorophenyl)2-[4,5,6,7-tetrahydrothieno(3,2-c)pyridine] acid chloride and 0 .2 g salicylic acid and 1.5 ml of dry pyridine was taken. The reaction was refluxed gently on bath for 40-50 min. Then the solution was allowed cooled, filtered and the crude of white granule was collected.

I. 2-(2-chlorophenyl2-[4,5,6,7-tetrahydrothieno(3,2-c)pyridine]acetosalicylic acid

Yield 60%; mp 170-172^{O}C (Isopropyl alcohol). –IR[ν, cm^{-1},KBr]: 2630 (S-H),1850 (C-OH),1700 (C=O),2500 (N-H),800 (C-Cl),3122 (ArC-H) 1HNMR(300Hz,CDCl3)δ7.091(3,1H J=3.531) 7.252(4, 1H J=8-091); MS, m/z; 440(M+1).

II. 2-(2-chlorophenyl)2-[4,5,6,7-tetrahydrothieno(3,2-c)pyridine]aceto3-bromosalicylic acid

Yield 55%; mp171-173^{O}C (Isopropyl alcohol). –IR[ν, cm^{-1},KBr]: 2630 (S-H),1840 (C-OH),1700 (C=O),2500 (N-H),800 (C-Cl),3122 (ArC-H),530 (C-Br) 1HNMR(300Hz,CDCl3)δ7.454(3,1H J=5.377); MS, m/z; 519(M+1).

III. 2-(2,4-dichlorophenyl)2-[4,5,6,7-tetrahydrothieno (3,2-c)pyridine]acetosalicylic acid

Yield 55%; mp 171-173^{O}C (Isopropyl alcohol). –IR[ν, cm^{-1}, KBr]: 2630 (S-H),1845 (C-OH), 1700 (C=O),2500 (N-H),750 (C-Cl_2),3120 (ArC-H) 1HNMR(300Hz,CDCl3)δ7.2(3, 1H J=4.943); MS, m/z; 475(M+1).

IV. 2-(2,4-dichlorophenyl)2-[4,5,6,7-tetrahydrothieno(3,2-c)pyridine]aceto3,5 dibromo salicylic acid

Yield 50%; mp 171-174^{O}C (Isopropyl alcohol). –IR [ν, cm^{-1}, KBr]:2630 (S-H),1850 (C-OH),1700 (C=O),2500 (N-H),750 (C-Cl_2), 3120 (ArC- H),800 (C-Br_2) 1HNMR(300Hz,CDCl3)δ7.4(6, 1H J=); MS, m/z; 633(M+1).

Pharmacological study

Antiplatelet and antithrombotic activity

Procedure

Antiplatelet and antithrombic activity was performed by administration of test drugs at the doses (10mg/kg, 30mg/kg) equivalent to clopidogrel by suspending in 1% Tween 80 solution. One group was kept as control and received oral administration of 1% Tween 80% solution. Clopidogrel and Aspirin were used as reference drugs. Bleeding time was observed by tail transaction bleeding time method. Derivatives are orally administered 4 h before transaction under anesthesia with isothiopental sodium (45 mg/kg I.P), the rat tail was transacted at 4 mm from the tip by a scalpel and the tail was immediately immersed into warmed saline (37%) until blood flow stopped. Bleeding time assessed as the time from tail transaction to the termination of blood flow. Bleeding time of mutual prodrugs were compared with standard drugs the results were given in Table: 2.

RESULT AND DISCUSSION

A series of mutual prodrug of clopidogrel and aspirin substitute were synthesized using appropriate synthetic route. The mutual prodrugs were further screened physicochemically and pharmacologically. All synthesized compounds are appeared as white crystalline powder and solubility was found in methanol. The preliminary characterization data of synthesized compounds were given in Table: 1.

Table 1: Physicochemical data of Synthetic Mutual Prodrug.

Comp Code	R_1	R_2	Mol.formula	Mol. wt	M.P	R_f value	% Yieid
I	2-Cl	H	$C_{23}H_{20}ClNO_4S$	441.5	170-172 ^OC	0.52	60
II	2-Cl	3-Br	$C_{22}H_{17}ClBrNO_4S$	520.5	171-173 ^OC	0.57	55
III	2-Cl,4-Cl	H	$C_{22}H_{17}Cl_2NO_4S$	476	170-173 ^OC	0.55	55
IV	2-Cl,4-Cl	3-Br,5-Br	$C_{22}H_{15}Cl_2Br_2NO_4S$	634	171-174 ^OC	0..61	50

FTIR spectra of all compound shows aromatic C-H stretching vibration 3121cm^{-1} indicates that the C-H bond present in aromatic ring. All derivatives showed a broad absorbance band at about 2500-2550 cm^{-1} associated with stretching vibrations of bonded N-H, indicating presence of nitrogen. Every compound showed a strong absorbance band due to C=O stretching vibration 1700 cm^{-1}. Compound-1 showed a strong absorbance 800 cm^{-1} stretching vibration indicating present of Cl group. Compound-2 shows absorbance at 530 cm^{-1}stretching vibration indicating present of Br group. Compound-3 shows C-Cl_2 stretching vibration at 750 cm^{-1} indicating present of dichloride group. Compound-4 shows C-Br_2 stretching vibration at 800cm^{-1} indicating present of dibromo group. This is further confirmed by NMR and MASS spectra. ^{1}NMR shows a sharp peak at 10.2 ppm, indicating present of acid group. Aromatic hydrogen shows peak at 6-7.5 ppm. Mass studies show M+2 peak indicating present of chlorine and bromine in compound.

Bleeding time was determined from the time of tail transaction to the termination of blood flow. Bleeding times beyond 3250 seconds were compared to standard drug clopidogrel, aspirin, and clopidogrel plus aspirin. Bleeding time was 300±5.774 seconds (mean ± S.E) without drug treatment. After an oral administration of mutual prodrug at 10 mg/kg, the bleeding time was remarkably prolonged to 2458 seconds. In the case of 30 mg/kg as mutual prodrug, the bleeding time reached the maximal detection point 3250 second. All compounds shows better activity compared to standard drugs and physical mixture of clopidogrel and aspirin. The compounds I to IV showed increased antiplatelet and antithrombotic activity as compared to standard drug.

Table 2: Bleeding time of mutual prodrug after 10mg/kg and 30mg/kg dose.

Group no.	Compound specification	Bleeding time (Sec)Mean ± S.E	
		10mg/kg	30mg/kg
I	Control	300.00 ± 5.774	300.00 ± 5.774
II	Aspirin	480.00 ± 1.145	1350.0 ± 0.445
III	Clopidogrel	1700.0 ± 1.155	1800.333 ± 0.882
IV	Clo. + Asp.	2250.00 ± 1.250	2855.00 ± 1.345
V	Comp. I	2300.667 ± 1.764	2980.667 ±1.453
VI	Comp. II	2442.667 ± 1.453	3200.333 ± 0.882
VII	Comp. III	2351.333 ± 0.882	2985.333 ± 1.453
VIII	Comp. IV	2458.000 ± 1.155	3250.333 ± 1.202

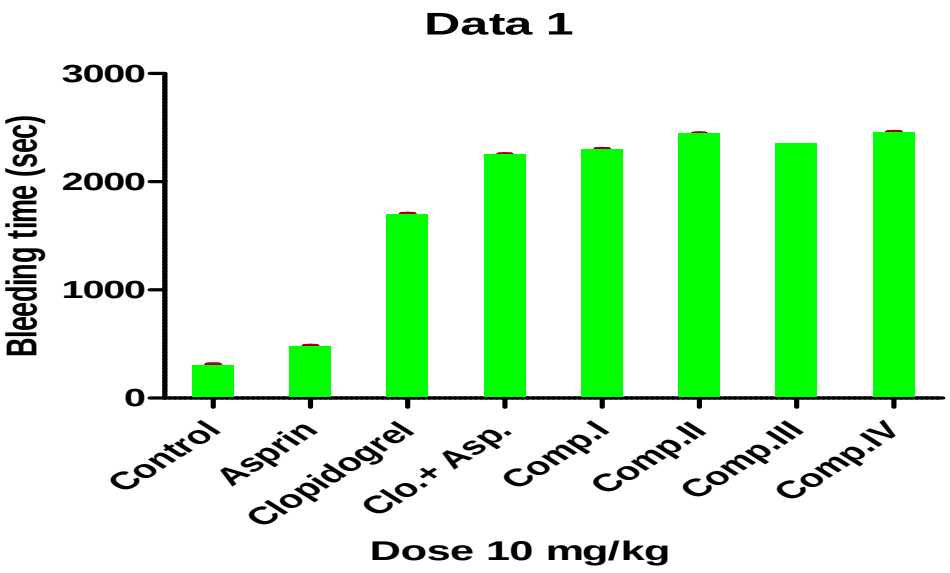
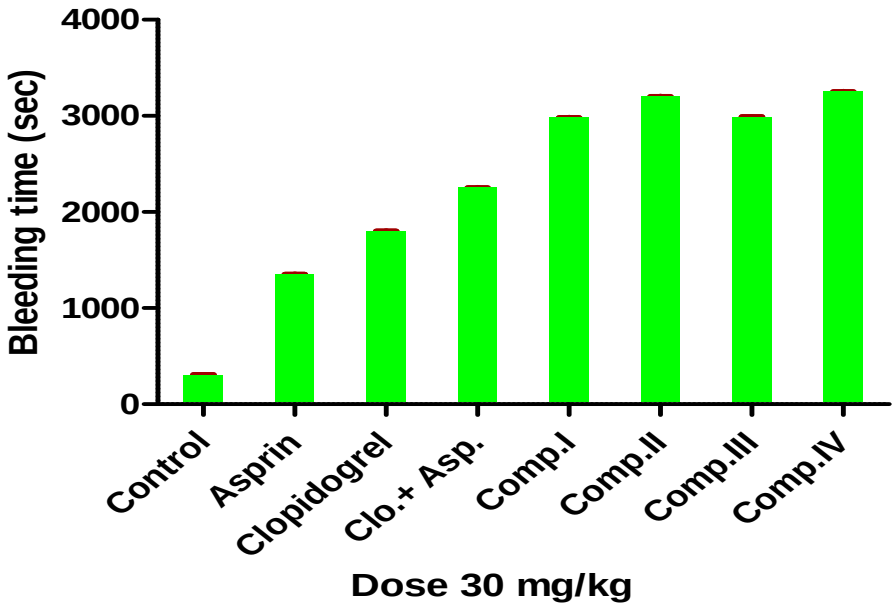


Table 6: Bleeding time of mutual prodrug after 30mg/kg dose.

Group no.	Compound specification	Bleeding time(Sec) mean±S.E
I	Control	300±5.774
II	Aspirin	1350±0.445
III	Clopidogrel	1800.333±0.882
IV	Clo. + Asp.	2855±1.345
V	Comp. I	2980.667±1.453
VI	Comp. II	3200.333±0.882
VII	Comp.III	2985.333±1.453
VIII	Comp.IV	3250.333±1.202



RESULT AND DISCUSSION

A series of mutual prodrug of clopidogrel and aspirin substitute were synthesized using appropriate synthetic route and screened for antiplatelet and antithrombotic activity. All synthesized compounds are appeared as white crystalline powder and solubility was found in methanol. All compounds shows better activity compared to standard drugs and physical mixture of clopidogrel and aspirin. The compounds I to IV showed increased antiplatelet and antithrombotic activity as compared to standard drug (Clopidrogrel).

CONCLUSION

The present research work aimed to develop mutual prodrugs from clopicogrel and aspirin has successfully carried out. The encouraging results of this dissertation work has definitely open a new way into this prodrug arena. In future this will definitely pick several new molecules which will play an important role in therapy of blood related disorders.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. D. R. Chaple Principal of Priyadarshini J.L. College of Pharmacy, Nagpur for providing lab facilities for research work.

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